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CONTENTS

	Page
Science for Scientists and Technologists	311
Goals for Science in the United States	312
Rotation of the Earth. By Prof. S. K. Runcorn	313
Astronomy in the Making. By Colin A. Ronan	314
Theoretical Meteorology. By Dr. Robert P. Pearce	314
Incompressible Aerodynamics. By Prof. Leon Trilling	315
Infra-red Arts. By Dr. E. S. Waight	315
The Electron Microscope. By Prof. D. Gabor, F.R.S.	316
Organo-Metal Compounds. By Dr. D. S. Payne	316
Radiation Chemistry of Organic Compounds. By Dr. S. J. Thomson	317
Physical Methods of Organic Chemistry. By Dr. W. A. Waters, F.R.S.	317
Chemistry and the Nervous System. By Dr. R. V. Coxon	318
Fluorine Chemistry. By Dr. Richard Dresdner	319
Natural Radioactivity in Tobacco and Tobacco Smoke. By Dr. V. C. Runeckles	322
Climate and Weathered Karroo Dolerites. By Dr. H. H. Weinert	325
Magnetic Storms in Interplanetary Space as observed by *Pioneer V*. By Eugene W. Greenstadt	329
Obituaries :	
Prof. W. T. Astbury, F.R.S. By Dr. I. MacArthur ; Dame Kathleen Lonsdale, D.B.E., F.R.S.	331
Mr. W. V. Lewis. By Mrs. Jean M. Grove	332
Mr. Geoffrey Parr. By T. H. Bridgewater	332
News and Views	333
Hazards of the Animal House	339
Use of Animal Cell, Tissue and Organ Cultures in Radiobiology. By Dr. Ilse Lasnitzki	340
The National Chemical Laboratory	342
The National Research Development Corporation	343
Fœtal and Neonatal Physiology	344
Life and Scientific Work of Sir James Hall	345
Hospital In-Patients in Britain	345
Origins of Petroleum	346
New Afterglow Emission associated with Metastable Nitrogen Atoms. By L. Herman and R. Herman	346
Chemisorption at Solid-Liquid Interfaces. By A. R. Burkin and G. Halsey	348
Modified Cellulose Ion Exchangers and their Use in Ribonucleic Acid Fractionation. By Dr. L. Bosch, Miss G. van der Wende and Dr. H. Bloemendal	349
Dimer Formation and the Transfer of Glycerol across the Erythrocyte Membrane. By Dr. W. D. Stein	352
Solubility of Denatured Hæmoglobin Variants in Acid Buffers. By I. D. Fraser and Dr. A. B. Raper	355
Thyroglobulin. By Dr. A. D. Litonjua	356
Correlation between Growth-rate of the Suckling of Various Species and the Percentage of Total Calories from Protein in the Milk. By Dr. F. W. Bernhart	358
Treatment of Hops (*Humulus lupulus*, L.) with Gibberellic Acid. By R. Stevens, J. B. Roberts and I. H. Williams; I. H. Williams	360
Chromatographic Investigation of the Methyl Alcohol/Chloroform-soluble Constituents of Honeybee Larval Foods. By N. G. Patel, M. H. Haydak and R. Lovell	362
Nervous and Biochemical Disturbances following Hexachlorobenzene Intoxication. By Dr. F. De Matteis, B. E. Prior and Prof. C. Rimington, F.R.S.	363
Mechanism of Sensitivity to Hydrostatic Pressure in the Prawn, *Palaemonetes varians* Leach. By Peter S. B. Digby	366
Location of Polygenes. By Prof. J. M. Thoday	368
Preparation of Mitochondria from the Peel of Apples. By Dr. J. D. Jones and Dr. A. C. Hulme	370
An Australopithecine Scoop made from a Right Australopithecine Upper Arm Bone. By Prof. Raymond A. Dart	372

(*Continued overleaf*)

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LETTERS TO THE EDITORS

Page

PHYSICS

Absorption Spectrum of γ-Irradiated Lead Silicate Glass.—R. S. Barker, T. K. Harrison, D. A. Richardson and R. Rimmer 374

Rotational Analysis of Absorption Bands of Lead Monoxide.—Dr. R. F. Barrow, J. L. Deutsch and D. N. Travis 374

Kirkendall Effect in Gases.—Prof. E. A. Mason, L. Miller and P. C. Carman 375

ENGINEERING

Effective Contact Stresses and Friction.—Dr. D. H. Trollope 376

CHEMISTRY

Quantitative Estimation of Peak Areas in Gas-Liquid Chromatography.—Dr. K. K. Carroll . . . 377

Compressibility and Specific Evaporation Resistance of the Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers.—A. V. Deo, S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas . . . 378

Electronic Properties of Imidazole.—S. Aftergut and G. P. Brown 379

Formation of Boron Nitride in Diborane–Hydrazine Flames.—W. G. Berl and W. E. Wilson . . . 380

Determination by Mass Spectrometry of the Structure of Proline Derivatives from Apples.—Dr. K. Biemann, G. G. J. Deffner and Prof. F. C. Steward, F.R.S. . 380

Determination of Lead in Urine by Atomic Absorption Spectroscopy.—J. B. Willis 381

BIOCHEMISTRY

Electrophoretic and Immuno-electrophoretic Patterns of Extracellular Diphtheria Proteins from Culture Filtrates of Different Iron Concentrations.—Bertold J. Radola 382

Formation of 5-Hydroxytryptamine in an Aqueous Solution of 5-Hydroxytryptophan treated with X-rays.—M. Jovic and Z. Supek 383

Lanthionine Formation in Keratin.—Dr. C. Earland and D. J. Raven 384

Effect of Polyamino Carboxylic Acids on Catalase.—Dr. Joan Bond and J. G. Cooper 385

Dextransucrase *V* and the Role of Metal Ions in Enzyme Catalysis.—W. Brock Neely and Julie Hallmark . 385

Degree of Polymerization of Hyaluronic Acid isolated from Human Aortic Tissue.—Dr. Sv. Bertelsen and K. Marcker 386

Incorporation of Deoxyribonucleic Acid by Mammalian Cells *in vitro*.—Prof. E. R. M. Kay . . . 387

Anticancer Properties of Thio-diglycollic Acid.—M. B. Sahasrabudhe, M. V. Narurkar, L. B. Kotnis, B. D. Tilak, L. C. Shah and V. N. Gogte 388

HISTOCHEMISTRY

Muscle Target Fibres, a Newly Recognized Sign of Denervation.—Dr. W. K. Engel 389

RADIOBIOLOGY

Assessment of Chronic Exposure to Radiostrontium by Urinary Assay.—Dr. C. L. Comar and Jay Georgi . 390

Metabolism of the Sulphur Amino-Acids and of Sulphate in *Blattella germanica*.—Richard J. Block and S. Mark Henry 392

PHYSIOLOGY

Selective Uptake of Specifically Bound Cobalt-58 Vitamin B_{12} by Human and Mouse Tumour Cells.—Dr. Bernard A. Cooper and Dr. William Paranchych 393

Outflux of Inorganic and Organic Phosphate during Membrane Depolarization of Excitable Tissues.—Dr. L. G. Abood, Prof. K. Koketsu and I. Koyama . 395

Page

A Hydrostatic Function of the Accessory Respiratory Organs in Air-breathing Fishes.—Dr. S. Z. Qasim and R. Hasan 396

HÆMATOLOGY

Abnormal Fœtal Hæmoglobins.—Prof. E. Silvestroni and I. Bianco 397

Thalassæmia and the Abnormal Human Hæmoglobins.—Dr. Harvey A. Itano and Prof. Linus Pauling, For.Mem.R.S. 398

PATHOLOGY

Anaphylactoid Reaction in Rats.—J. M. Harris and Dr. G. B. West 399

Treatment of Thrombocytopænic Hæmorrhage with Œstriol Succinate.—Dr. Hubert Poliwoda . . . 400

Effect of Enzyme Injections on Mitosis in Regenerating Liver.—Dr. Gaston de Lamirande 400

BIOLOGY

Myopathy affecting the Rottnest Quokka (*Setonix brachyurus*) reversed by α-Tocopherol.—B. A. Kakulas 402

Structure and Action of the Sucker of *Echeneis*.—Prof. B. Bonnell 403

The Path of the Giant Cell Axons in *Aplysia depilans*.—Dr. G. M. Hughes and L. Tauc 404

Maxillary Index in *Anopheles gambiae* Giles.—L. K. H. Goma 405

Rectal Glands in Woodlice.—Dr. Minati Gupta . . 406

Mechanism of Differential Staining of Nucleic Acids.—D. J. Goldstein 407

Dispersal of Tropical Seeds by Ocean Currents.—Miss R. Mason 408

Classification of the Tea Plant.—Dr. H. H. Mann . 409

Staining Reaction of the Pit Membrane of Wood Cells.—R. K. Bamber 409

A Sealing Compound for use in Biological Work.—G. Stotzky, W. Culbreth and Dr. L. B. Mish . . 410

MICROBIOLOGY

Activation of Amino-Acids in Micro-Organisms which produce Antibiotics.—Dr. O. Ciferri, Dr. M. Di Girolamo and Dr. A. Bendicenti Di Girolamo . . 411

Enzymic Binding of Hydroxylamine by Fumaric Acid.—Prof. Nathan Grossowicz and (the late) Yehudith Lichtenstein 412

Induced Increase in Resistance of *Entamoeba histolytica* to Fumagillin.—Dr. Mitsuru Nakamura . . . 413

VIROLOGY

Effect of *p*-Fluorophenylalanine on the Multiplication of Foot-and-Mouth Disease Virus.—F. Brown, D. N. Planterose and Doreen L. Stewart . . . 414

Leaf Curl Virus and the Flavonoid Content of Resistant and Susceptible Strains of Cotton.—Dr. Esam M. Moustafa 415

SOIL SCIENCE

Isolation of Anthraquinone from Humus.—Kyoichi Kumada, Akio Suzuki and Kazuyuki Aizawa . . 415

PSYCHOLOGY

Measuring Visual Constancy for Stationary or Moving Objects.—S. M. Anstis, C. D. Shopland and R. L. Gregory 416

ANTHROPOLOGY

The Juvenile Mandible from Olduvai.—Dr. L. S. B. Leakey 417

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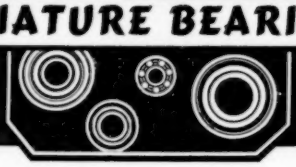

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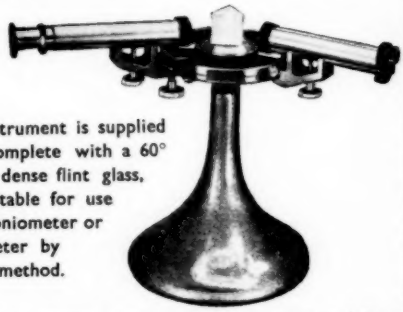

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SCIENCE FOR SCIENTISTS AND TECHNOLOGISTS

PRE-OCCUPIED as they are with problems of providing sufficient quantities of concrete and glass to accommodate all the pressing applicants, university leaders have found it difficult of late to spare the time to consider the kind of teaching which will best serve the graduate scientist or technologist. In his statement to Convocation of the University of Leeds, the vice-chancellor, Sir Charles Morris, wisely resisted the temptation to deal with the expansion difficulties of the 'sixties and concentrated on the problems which are immediately in front of all university teachers and administrators and which all can help to solve.

Chief among these is the kind of science which should be included in undergraduate courses for scientists and technologists. Despite the accelerating pace of scientific discovery, the problem of adjusting syllabuses to meet the needs of students of pure science are, though difficult, not insuperable. Although much that was considered essential in the past necessarily has to be cut out, at regular intervals there is a rethinking of the principles on which courses and syllabuses have to be rebuilt, and this is done at not infrequent intervals.

With the applied sciences however, and this applies to medical as well as technological courses, the situation is quite different. Here knowledge of a pure science is not an end in itself. It is a piece of equipment which is necessary before the study of the applied science or the acquirement of professional skills can be initiated. The underlying danger is that the amount of knowledge of the pure sciences which teachers of technology demand may be much more than individual students can humanly be expected to absorb.

Nor are the difficulties confined to the students. Studies undertaken by pure scientists in teaching the applied scientists are becoming so exacting that the prospect of the pure science departments themselves becoming considerably changed in nature and direction is by no means remote. The prospective engineer or metallurgist or medical man regards his basic chemistry, physics and mathematics as so many obstacles to be got over before embarking on the studies that matter. Teaching physics or chemistry to such students involves much routine work and, to sustain student interest, demands not only much pedagogical skill but also a considerable quantity of evangelical zeal. To maintain continual interest, the teacher may have to resort to methods of teaching which may not always have been thought appropriate in university departments.

This is a problem which needs speedy resolution if departments of pure science are to continue to flourish both as centres of creative investigation and places where pure scientists themselves may be taught. Sir Charles Morris believes that if this problem is not solved, "the departments of pure science as centres for the advancement of knowledge and homes for education of the pure scientists of the future, may in many universities wither and die". The alternative is that our future doctors and technologists may be brought up with insufficient knowledge of the basic sciences on which the whole structure of medicine and technology rests.

To push this problem back to the schools would provide no lasting solution. Already the fear exists that, so far as science is concerned, too much is being demanded of the schools. Since the amount of pure science required by the technologists will grow more rather than less, it would seem inevitable that the universities will be required to teach more pure science to the applied scientists.

The content of courses for technologists was also examined by Sir John Cockcroft, Master of Churchill College, Cambridge, in his Trueman Wood Lecture delivered to the Royal Society of Arts on April 26. Since their training should enable them to deal with the technological developments of the future rather than the problems of the past, Sir John suggested that university courses in technology should have a strong component of basic science. All students of technology, for example, should become familiar with the fundamentals of electricity and magnetism so that they can design equipment from first principles rather than handbooks. They should also know the principles of heat and thermodynamics, fluid dynamics as well as solid-state physics, which form the basis for understanding those solid-state electronic devices which are changing the face of technology. All basic training should stem from a good working knowledge of mathematics, particularly calculus and differential equations. Sir John would find wide support for his contention that mathematics should take priority over all other subjects in the training of sixth-form scientists who wish to enter a university or go directly into industry. Many would also support his view that to-day there is too much of a divorce between mathematics and physics in some universities, but would hesitate in replacing mathematics entirely by theoretical physics in the training of technologists.

In the first one or two years of university education, potential technologists should study physics, chemistry and mathematics, and Sir John commends as a model the Cambridge Natural Sciences Tripos Part I in which three or more sciences, including mathematics, are studied.

The training of engineers should follow the same general principles that apply to the training of other technologists. In his first two years, the engineer should study those principles of engineering science which underlie his technology. Only then should he begin to specialize and spend a large part of his time

on electronics, thermodynamics, power engineering or other specialist subjects. Support for Sir John's thesis came recently from the Institution of Chemical Engineers, which recommended that about 45 per cent of the teaching in a university should be devoted to pure science and mathematics. About an equal amount of time should be devoted to chemical engineering science and the remainder to other engineering science, report-writing and a foreign language.

Regard for the way basic science is being taught in Australian universities has been expressed by Prof. A. E. Alexander, professor of physical chemistry in the University of Sydney (*Vestes*, **3**, No. 4; December 1960). Prof. Alexander's reflexions were mainly concerned with chemistry and stem largely from the low staff–student ratio. At the University of Sydney enrolments for chemistry in 1960 were 2,250 and were accommodated in eight parallel lecture courses, these being taken by three lecturers, two temporary lecturers, and three teaching Fellows. In Australia little sixth-form chemistry is taught in the schools and Prof. Alexander sighs for the British system so that the university could be spared an intolerable burden of mass production and be able to spend more time on teaching chemistry more appropriate to a university (see also *Nature*, **189**, 444, 1961). For some time, however, the problem of large student numbers has to be faced and raises anew the questions posed by Sir Charles Morris—what kinds of basic science should be taught to applied scientists, how much of it should be taught in the schools and the university, and what teaching techniques the universities should adopt to deal with the inevitable large numbers. American universities have paid much more attention to what should be taught and how it should be taught, and both Britain and Australia have much to learn from them.

The time has surely come when such fundamental decisions should no longer be left to heads of departments or *ad hoc* committees composed of members whose main interests are in research. Should not each faculty of pure and applied science initiate major research projects, carried out by senior research Fellows, to determine the content of their courses, the way it should be taught, where the teaching should be done, how much should be left to the schools, how often revision should be carried out, and who should be consulted before revision takes place?

GOALS FOR SCIENCE IN THE UNITED STATES

IN February 1960, General (formerly President) Eisenhower announced the appointment of a Commission on National Goals, and requested the members of this Commission to "develop a broad outline of co-ordinated national policies and programs" and to "set up a series of goals in various areas of national activity". The Commission's report, including sixteen essays written by selected individuals, was prepared and published in a volume entitled *Goals for Americans*.

For its essay on science, the Commission chose Dr. Warren Weaver, vice-president of the Alfred P. Sloan Foundation, who, aided by the advice and counsel of a number of distinguished American scientists, presented his contribution, which he called "A Great Age for Science".

As this article was written by one of America's leading scientists, and because of the number of requests for the material, it has been reprinted separately by the Foundation for special distribution*.

Of primary importance in Dr. Weaver's thesis is the need to recognize the importance of science teaching, and to reward teachers at all levels of the educational system. The fact that this need has been appreciated for some time, and is fairly self-evident, does not detract from its intrinsic importance. Concern is growing in the United States about the present-day neglect and disparagement of teachers, which, long-existent at the more elementary levels, has now obtained a foothold in the universities. The university tradition of great scholar-teachers, dedicated to research, but eager and willing to teach younger students, is being undermined. Nowadays scientists are being offered research posts with the added 'advantage' of very little teaching duty, and many full-time research scientists, working at the universities and receiving salaries from research contracts and grants, are, in the words of Dr. Weaver, "intellectual eunuchs, not at all involved in the propagation of their kind". Furthermore, the premium placed on particular types of scientists for certain industries (notably the defence industry) often leads to emergent Ph.D.s being offered research posts with salaries exceeding those of the professors supervising the theses. These trends are tending to denigrate the academic dignity and fundamental importance of teaching, and the trend should be reversed. It is Dr. Weaver's contention that there are no secondary roles so far as teaching and research are concerned; they should be equal partners.

Much space is devoted to a discussion of the position of science within the framework of society. That an enormous gap has opened up between the thinking of scientists and the views and attitudes of mind of creative artists and moralists is serious enough, but even more disastrous is the chasm which separates the scientist from the layman. Dr. Weaver quotes from *The New Yorker* of March 5, 1960: "These are hard times for the layman. He is no

* *A Great Age for Science*. By Warren Weaver. Pp. viii + 35. (Reprinted from *Goals for Americans*.) (New York: Alfred P. Sloan Foundation, 1961.)

longer thought competent to work out his own opinions on many matters, even many that touch him intimately. His very survival has become the property of committees and the subject of learned arguments among specialists. He has little to say . . . being largely ignorant of the information upon which plans for him are based".

Science is involved in many present-day social and political problems, and, in a democracy, these problems should be the concern of the citizens as well as the scientists. Further to this, science in the United States is now largely financed by Government agencies, which are under the ultimate control of the citizens. People ought not to have to subscribe to something they know nothing about.

These points make it imperative that individual citizens should have an improved understanding of what science is, and how it operates.

On more specific but nevertheless highly important goals, the question of financial support for science is discussed. Often there is not enough distinction made between research and development, and a cursory glance at budget statements of 'R and D' (Research and Development) may give a misleading impression of the amount of support given to 'R'. The figures indicate an enormous increase in the support of development, but the financial aid given to basic research has not kept pace. Although American defence commitments require enormous sums to be spent on development of missiles, rockets, etc., there is some criticism of the fundamental philosophy of State policy in this direction; military interservice rivalry and lack of understanding of military objectives lead to wasteful duplication of developmental projects, and 'crash programmes', undertaken more as a result of emotion than of reason ('space' is the perfect example), lead to a budget which is heavily overburdened on the developmental side. It is argued that, with the introduction of a little more common sense, the total annual national allotment for pure research, which at the moment is in the region of 8–900 million dollars, could easily and profitably be raised to 1,500 million dollars without increasing the overall research and development figures.

Attention is also directed to the part played by industry in promoting basic scientific research. Although most of the misguided, old-fashioned acrimony between academic and industrial scientists has now disappeared, only a very few of the best American industries have established a satisfactory liaison between the interests of the community and the interests of the individual. Other industries, although giving the appearance of following this pattern, do not, in fact, go far enough. Furthermore, although there has been great improvement, industries do not, in general, give enough support to research and education in universities and colleges, or to development of particular fields of science on which they themselves depend.

'Crash programmes', which work on the false assumption that 2 billion dollars equal one solved cancer problem, often lack adequately trained and able personnel, and, most important of all, there has not been established a basic theory to work on. What is needed is a broad attack at the roots of a problem by means of reasoned, fundamental research rather than a phrenetically active but superficial attack, which reflects adversely on national maturity and prompts Dr. Weaver to ask himself, "What's the rush ?"

ROTATION OF THE EARTH

The Rotation of the Earth

A Geophysical Discussion. By Walter H. Munk and Gordon J. F. Macdonald. (Cambridge Monographs on Mechanics and Applied Mathematics.) Pp. xix+323. (Cambridge: At the University Press, 1960.) 70*s*. net.

THIS masterly book very well conveys the intellectual excitement of present-day geophysical research. From an examination of the places from which ancient eclipses were observed, Halley pointed out that the Moon's motion in longitude was gradually accelerating. Some of the best mathematical minds in the eighteenth century concerned themselves with the complex problem of providing a complete theory of the motions of bodies of the solar system, and Laplace in 1787 finally decided that this acceleration of the Moon was due to a decrease in the eccentricity of the Earth's orbit arising from gravitational perturbations by the planets. However, about the middle of last century, J. C. Adams showed that Laplace had neglected certain terms and that, in fact, only half the acceleration of the Moon could be explained by purely gravitational causes.

Gradually it became evident that the discrepancy was due to a secular increase in the length of the day, that is, a slowing up of the Earth's rotation. The much more detailed knowledge of the Moon's longitude, acquired through astronomical measurements in the past few hundred years, has shown that in addition to this secular acceleration of the Moon's longitude there are irregular changes with a time scale similar to that of the geomagnetic secular variation (about 50 years). The work of numerous astronomers, culminating in the famous paper of Spencer Jones in 1939, showed that these irregular fluctuations are also due to the changes in the length of the day. Since the War the Earth's rotation has been timed accurately over much shorter periods still by quartz clocks and an annual term in the Earth's rotation discovered.

About a hundred years ago astronomical determinations of the latitude of observatories became accurate enough to detect that the Earth's axis of rotation is wobbling, with respect to the Earth, a few tens of feet during periods of twelve months and fourteen months. The extraordinary accuracy of astronomical measurements by which these various phenomena have been discovered should be a cause of wonder: the irregular changes in the length of the day, for example, are about one part in 10^8. All these phenomena give very important information about the Earth, and Munk and Macdonald give a fascinating description of the analysis required to explain them. The secular deceleration of the Earth appears to result from the friction of the lunar oceanic tides; the irregular fluctuations in the length of the day from the turbulent motion

in the Earth's core, which produces the magnetic field; and the seasonal changes from asymmetry in the atmospheric circulation in the southern and northern hemispheres. The exciting forces which cause the Earth to wobble on its axis are unknown, and this causes some difficulty at present in making reliable inferences concerning the properties of the interior of the Earth from the variation of latitude data.

However, on the whole, all the observations of phenomena so far referred to can be explained without having to assume any very startling new departures in geophysical thinking, and so, as Munk and Macdonald show, a mathematically satisfying and fairly tidy story results. However, for the completeness of their account of the idiosyncrasies of the Earth's rotation, Munk and Macdonald refer to the evidence, which has accumulated in recent years from palæomagnetic work, that large changes of the relative positions of the axis of rotation and the continents have occurred gradually over hundreds of millions of years. These theories of 'polar wandering' and 'continental drift' were vigorously debated thirty years ago by geologists and geophysicists and were then largely discredited. Munk and Macdonald discuss the possibility of producing large polar wandering, but they baulk at the idea of continental drift. Instead, they take refuge in a somewhat unsympathetic discussion of the palæomagnetic evidence, although these data are massive in quantity and are probably more statistically significant than some of the other observational evidence which they accept quite happily in the earlier part of their book. The beauty of the book is to show how evidence from such widely different fields as geology and positional astronomy must be woven together to understand this central problem of geophysics.

S. K. Runcorn

ASTRONOMY IN THE MAKING

The Controversy on the Comets of 1618

By Galileo Galilei, Horatio Grassi, Mario Guiducci and Johann Kepler. Translated by Stillman Drake and C. D. O'Malley. Pp. xxv+380. (Philadelphia: University of Pennsylvania Press; London: Oxford University Press, 1960.) 48*s*. net.

As the links of the new astronomy were being forged in the seventeenth century, controversies, often heated and sometimes polemical, arose among the *cognoscenti*. On one hand the Aristotelian and Ptolemaic universe was upheld, having the respectability of authority and the blessings of the Church behind it; on the other the revolutionary ideas of Copernicus and Tycho Brahe were advocated by Galileo and Kepler, who also had their own novel contributions to make and who were supported by others of like mind. One of the difficulties of looking back on these formative years is so to stimulate the imagination that one can obtain something of the essence of the reality of the problems which had to be solved. It is only too easy to judge with a decision based on what we now know was later to be discovered and proved. Probably the best way out of this difficulty is, where possible, to read the actual arguments which took place and, moreover, to read them in the words of the actual protagonists concerned.

In the autumn of 1618 there appeared three bright comets, visible to the naked eye. The question arose whether these objects were meteorological in nature or whether they were truly celestial bodies and, if the latter, what really was the nature of their paths. The appearance of the comets aroused wide interest, many pamphlets about them appeared, and Galileo, then aged fifty-four, entered the arena of controversy and produced his brilliant polemic *Il Saggiatore*. This book is important not so much for its scientific content or its literary merit, high though this is, but primarily for an insight into Galileo's approach to scientific matters; modern scientific method is here seen as it was in its early stages.

Yet Mr. Drake and Dr. O'Malley have, in their book, done more than provide, for the first time, a translation of the whole of *Il Saggiatore*, for they have also placed this gem in its appropriate setting. This they have achieved by beginning with a translation of Gassi's disputation on the three comets and giving also his *Libra Astronomica*, which was published under the pseudonym of Lothario Sarsi. Interspersing Gassi's work is a translation of Guiducci's discourse on the comets (and really written by Galileo), and following Gassi's *Libra Astronomica* there is Guiducci's own letter about the matter written to his old professor, Tarquinio Galluzzi. *Il Saggiatore* comes next, and then there are appended Kepler's remarks on it taken from his *Tychonis Brahei Dani Hyperaspistes*. Thus, within the covers of one volume, we have the whole of the controversy over the three comets.

The works given are, of course, eminently readable, for their authors wrote well and the translations which have been made are excellent. Excellent also is Mr. Drake's nineteen-page introduction in which he sets out very clearly sufficient of the ramifications which lay behind the controversy and of the nature of the protagonists to make the translations illustrate their points clearly. Throughout the book there are references to notes which, given at the end of the volume, elucidate various points or obscurities in the main body of the text, and there is also an adequate index. Well printed in England, the price of the book may seem, perhaps, rather high, but nevertheless this excellent contribution to the history of science is much to be recommended.

Colin A. Ronan

THEORETICAL METEOROLOGY

Introduction to Theoretical Meteorology

By Prof. Seymour L. Hess. Pp. xiv+362. (New York: Henry Holt and Company; London: Constable and Co., Ltd., 1959.) 60*s*. net.

This is an important new text-book written primarily for students, but equally suitable for those desiring an up-to-date review of the subject. The aim has been to provide a reasonably rigorous treatment which is not rendered unnecessarily difficult by advanced mathematical arguments. The first seven chapters are devoted to basic thermodynamics, the properties of water vapour and hydrostatic stability, and the next three chapters to radiation theory and its application to the Earth-atmosphere system. The remaining eleven chapters deal with dynamics, including viscosity, turbulence and modern numerical techniques.

The main emphasis is placed on large-scale properties of the atmosphere. The general circulation and the role of depressions and anticyclones in

its maintenance receives a thorough treatment which brings the reader with a minimum of difficulty right to the frontiers of our knowledge of what is probably the most fascinating of all meteorological problems. On the other hand, smaller-scale phenomena such as sea-breezes are given a somewhat cursory treatment, and mountain waves are not mentioned at all, even though they have received much attention in recent years. Convection is discussed in more detail, but the impression given that a convection current behaves dynamically like a rising balloon subjected to viscous drag is misleading, since an isolated convection element is now known to be a rising vortex ring. The author's derivation of the equations of motion of the atmosphere is somewhat novel and will appeal to many, but may not satisfy those who are mathematically inclined. A more rigorous derivation, preferably using vectors, could well be appended.

These criticisms of detail must not be allowed to detract from the overall good quality of this book. The author's style is lucid and his presentation of some concepts, such as inertial stability and the vorticity equation, is particularly attractive. The examples at the end of each chapter are well designed and will help the student to acquire a knowledge of the factors which are of primary importance in the many and varied physical problems encountered in meteorology.

ROBERT P. PEARCE

INCOMPRESSIBLE AERODYNAMICS

Incompressible Aerodynamics

An Account of the Theory and Observation of the Steady Flow of Incompressible Fluid past Aerofoils, Wings, and other Bodies. Edited by Bryan Thwaites. (Fluid Motion Memoirs.) Pp. xx+636. (Oxford: Clarendon Press; London: Oxford University Press, 1960.) 75*s*. net.

THIS book is the first of a new series of Memoirs on Fluid Motion which will also include volumes on laminar boundary layers and turbulence. It addresses itself to a class of problems which have been pushed off the centre of the aerodynamics stage by the current fashion for magnetogasdynamics, hypersonics, rarefied flows and all the other exotic fields associated with space flight or long-range rocketry—namely, the many non-linear problems of incompressible aerodynamics.

Nevertheless, these more old-fashioned problems of low-speed flight are neither irrelevant nor by any means solved or even fully understood, and the present volume is a welcome addition to the library of those in the aeronautical profession who are still concerned with aircraft which must take off, manœuvre and land with a reasonable degree of effectiveness and reliability; nor will it be found trivial by those many lovers of fluid mechanics who are fascinated by rotational and separated flow-patterns.

The basic thread which runs through this book is the subtle interplay of models of flow patterns suggested by ingenious experiments (both detailed measurements of pressures and visualization of flow fields by means of smoke, tufts and other devices) and approximate analytical techniques designed to fit the observed flow patterns into the fabric of classical aerodynamics. Never is the model oversimplified to make it amenable to analysis; never is mathematics pursued for its own sake to a sophistication beyond that of the physical picture. At all times, the relevance of the questions and of the answers to the design of more effective machines is clearly kept in mind.

Among the sections which I have found of particular interest, those which deal with stalling wings, separated flows over swept wings and thick wakes, appear outstanding; but this judgment is most likely a reflexion of my tastes. The treatment of almost all the difficult low-speed flight problems by ingenious methods is fascinating and a worthy monument to the men, largely at the National Physical Laboratory and the Royal Aircraft Establishment, who created a distinctive English style in aerodynamics, compounded of sound technique, balanced judgment and a profound intuitive feeling for the physics of fluid flow problems.

LEON TRILLING

INFRA-RED ARTS

Infrared Methods

Principles and Applications. By G. K. T. Conn and D. G. Avery. (Physical Chemistry: a Series of Monographs, Vol. 7.) Pp. viii+203. (New York: Academic Press, Inc.; London: Academic Press Inc. (London), Ltd., 1960.) 6.80 dollars

A SPECTACULAR change in the attitude of chemists over the past twenty years has been their acceptance of physical methods for solving problems, particularly in organic structural work and in analysis. Among these methods is infra-red spectroscopy, which is now, and is likely to remain, a routine tool of the chemist. The arrival of 'simplified' commercial instruments is making the infra-red spectrometer as commonplace in laboratories as the refractometer and is creating a class of user who has no interest in spectroscopy for its own sake and little in the principles of operation and design of instruments; infra-red spectroscopy is no longer the preserve of the physicist and physical chemist. Any book with the broad title *Infrared Methods—Principles and Applications* is thus likely to have drawn to it the attention of a very large number of students and research workers. It must be said, however, that many of these will find the title misleading, for the principles are those involved in generating, dispersing, and detecting infra-red radiation, the applications are in the calibration of detectors, the production of monochromatic radiation, in the design of instruments for industrial gas analysis and plant control, and in pyrometry. This book is written from the point of view of the experimental physicist, and when the authors suggest that it is an introduction to infra-red studies for "the research student and the student in allied fields" it should be understood that they refer to the research student of spectroscopy or allied fields of physics. The average worker in other fields who wants to understand how an infra-red spectrometer works and what its limitations are will find it far beyond his needs. Authors write as they view the subject, but it does seem remarkable to find a work such as this in a series of monographs on physical chemistry. There is, without doubt, a demand for an up-to-date authoritative account of practical methods in infra-red spectroscopy written with chemical applications strongly in mind. This monograph does not satisfy it.

The chosen material is presented in a clear manner with a fair number of references to original papers. The book is beautifully produced, only two errors being noted, in figures I. 2 and V. 5.

E. S. Waight

THE ELECTRON MICROSCOPE

The Electron Microscope

The Present State of the Art. By M. E. Haine, with a Chapter on Applications in Collaboration with Dr. V. E. Cosslett. Pp. xvi+282. (London: E. and F. N. Spon, Ltd., 1961). 55*s*. net.

THIS book, by the best British electron microscope engineer, with a chapter on applications written in collaboration with the doyen of British experts, is in many ways the last word on the electron microscope. It took the optical microscope 200 years to develop, from Leeuwenhoek to Abbe, from the 'dewdrop' to the apochromat. The electron microscope took less than 25 years from the primitive 'magnifying glass' of Knoll and Ruska, to the attainment of the present limiting resolution of about 6 Å., also by Ruska, in 1954, which, as readers of this book will soon become aware, is not likely to be much bettered in the future.

In the optical microscope two centuries of work went into correcting the aberrations, and the work is not yet quite finished. In electron optics it took only a few years before Scherzer demonstrated that the spherical aberration could not be corrected, and the question was only to find the optimum. In the electron microscope all the essential 'optical design' now boils down to a single curve (Fig. 1,7, p. 8), computed by the late G. Liebmann, one of the principal author's able collaborators, from which designers can pick their choice, according to the number of ampere turns which they are willing to embody in their objectives. The optimum theoretical resolution is about 2·7 Å. at 50 kV. and could be reduced to 1·5 Å. at 400 kV.—probably at the expense of contrast. The reasons why the practical resolution is only about one-half of the theoretical are fully and ably explained by Dr. Haine. It is particularly welcome that, apart from a detailed discussion of astigmatism, vibrations, creep, contaminations, etc., he describes, in full practical detail, the remarkable Haine-Mulvey Fresnel fringe test, so that every user of an electron microscope can test for himself the resolution limit of his own instrument.

The resolving power of the electron microscope has stopped at a particularly uninteresting stage. There are very few objects of 6 Å. size; organic molecules of this size have insufficient contrast, metal colloids tend to grow spontaneously to about twice this size, atomic lattice spacings are finer. (Only J. W. Menter was able to find an interesting example, by directly resolving the lattice of molybdenum trioxide, Fig. 10. 25, p. 270). If the resolution limit could be reduced below 3 Å., most atomic lattices could be resolved. The present book makes it clear why attempts to achieve this have failed, and are probably bound to fail for a long time—such as Scherzer's remarkable non-rotational lens, and the reviewer's suggestion of taking an imperfect electronic image, which is complete as it contains a record of phases as well as of amplitudes, and doing the corrections with light, in a second, optical process. The difficulties are so formidable that for a long time the electron microscope is bound to stay at the present limit—which, however, does not exclude interesting new special types, such as high-voltage instruments and interference microscopes, and the standard instrument will become a more and more convenient instrument for the user.

It is to the user—the biologist, the metallographer, the physical chemist—that this book is chiefly addressed. Correspondingly the mathematics is cut down to a minimum, but the critical attitude of Dr. Haine, who cannot suffer obscurities, has forced him to make this minimum so complete that it is somewhat doubtful whether most of the users of electron microscopes will not find it far above their heads. It takes a specialist to appreciate fully the amount of critical thinking which has gone into this book. I believe that it will have its greatest effect in the case of that small but important and growing class of users who are fully trained physicists (working mostly on imperfect crystals, such as metals) and who are willing to go to some pains to understand not only their object but also their instrument.

D. Gabor

ORGANO-METAL COMPOUNDS

Organo-Metallic Compounds

By Prof. G. E. Coates. Second edition. Pp. xiii+366. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1960.) 45*s*. net.

THE original stimulus for the investigation of organo-metallic compounds came from the possibility of their use in organic synthesis. Indeed, many chemists a decade or so ago were really familiar with only one class of compounds, namely, the Grignard reagents. To-day there exists an extensive subject covering compounds of almost all the known elements, which is rapidly developing and which is followed by many different kinds of chemist. The impressive advances which have been made have in many cases been the product of co-operation between theoretical, organic, inorganic and physical chemists. Synthetic organic chemistry is still, however, a major beneficiary from the investigation of these compounds, but there is an ever-increasing independence emerging in the studies in this field.

The first edition of this book (190 pages) appeared as a Methuen Monograph in 1956, having been written for the most part in 1953–54, with a section on the cyclopentadienyls having been added in proof. In the intervening years the amount of published work has been very great, so that even with certain condensations of material, notably that concerning organo-boron compounds, the second edition now comprises 360 pages and, in addition, has a larger page size. Such an expansion is inevitable and is completely justified in that this new and enlarged edition presents an almost complete picture of those compounds in which a direct metal–carbon bond is present. As in the previous edition the extensive organic chemistry of phosphorus, arsenic and silicon is omitted. The layout is little changed, but certain new theoretical and industrial aspects are now included. The main changes are to the sections on the alkali metals, boron, tin and the transition metals. This latter section has been expanded from 27 to 127 pages and now includes a thorough description of certain of the transition metal complexes in terms

of molecular orbital theory. Throughout, complexes are discussed in terms of σ or π bonding.

The author's personal enthusiasm and experience in this field are clearly apparent, particularly in certain of the descriptions of preparations in which considerable detail is given; an aspect which should add considerably to the value of the book for those actually working in the field. Others will find the wide coverage of this book most helpful in obtaining an appreciation of the present position of organometallics. The book is marred unfortunately by a number of typographical errors. The first edition was cheap at 12*s*. 6*d*. The second edition is reasonably priced at 45*s*. in view of its increased coverage.

D. S. PAYNE

RADIATION CHEMISTRY OF ORGANIC COMPOUNDS

Radiation Chemistry of Organic Compounds

By Dr. A. J. Swallow. (International Series of Monographs on Radiation Effects in Materials, Vol. 2). Pp. xiii+380. (London and New York: Pergamon Press, 1960.) 84*s*. net.

IN this book the author defines his objectives as the presentation of an overall picture of radiation chemistry and a review of the published work on the subject between 1895 and 1958. He begins with a brief introductory account of the interaction of radiation with matter, and a discussion of the transient species formed by radiation. The transient species are simple in the extreme compared with the highly complex systems to be discussed later in the book.

There follows a chapter on experimental methods which deals, only in outline, with radiation sources, purity of materials—a feature often overlooked, says the author, in some of the experimental work reported on—analysis of products, and dosimetry. In no sense is this a practical manual of techniques, but it surveys very adequately the background to the chapters which follow.

Very wisely, the author devotes the next chapter to the radiation chemistry of aqueous systems, partly because he can illustrate the fundamentals of the mechanism of radiation changes, and because of the importance of aqueous systems for the understanding of radiation effects on biological systems. The four chapters which follow deal with aliphatic and aromatic compounds, polymers and dyestuffs. In these chapters the author's task was difficult in the extreme for, as he says, mechanisms are not yet fully understood even for simple molecules. Thus, much of the material presented catalogues the results of radiation of a vast range of compounds, with comments on mechanism where this is even partially understood. I would have liked more comment on mechanism, no matter how speculative it had to be. The author deserves great credit for the thoroughness of his searches before presenting this—and the other material in the book.

The next chapter deals extensively with radiation effects on substances of biological interest, with limited reference to *in vivo* systems, and the final chapter deals with such diverse topics as the origin of organic compounds in the history of the Earth, radiotherapeutics, sterilization of pharmaceutical products, food, and detailed investigation of the economics of commercial radiation. This chapter contains the surprisingly few examples of commercially feasible radiation processes which have been discovered, in spite of the vast amount of irradiation experimentation in the laboratory. The importance of radiation damage to organic coolants and lubricants for reactors is also discussed.

Although it has a few stylistic defects, the book is written with great clarity, without unnecessary preamble or loquaciousness, in a refreshingly direct way. It covers an amazing range of material, which is referred to by a sensible reference system collected at the end of the book. The chapters are constructed according to well-established rules of learning—a brief statement of the aims of the chapter, the detailed discussion, then a brief statement of the results.

The main value of the book lies in the fact that it makes readily accessible the state of knowledge about particular systems. Its discussion of mechanism is necessarily brief; but since such a book as this in a subject which is rapidly growing will surely be revised, the author might consider greater elaboration of the discussion on fundamental aspects of the subject, whether the knowledge is speculative or tentative or otherwise, perhaps at the price of compressing some of the factual matter into tabular form. The present structure of the book reflects the way in which the subject has grown empirically, without a detailed and exact theoretical basis. Perhaps this situation will change in the cycle of experiment, theoretical interpretation, prediction, and further enlightened experimentation.

S. J. THOMSON

PHYSICAL METHODS OF ORGANIC CHEMISTRY

Physical Methods of Organic Chemistry

Part 3. Edited by Arnold Weissberger. Third, completely revised and augmented edition. (Technique of Organic Chemistry, Vol. 1, Part 3.) Pp. xii+1799–2634+13. 184*s*. Part 4. Edited by Arnold Weissberger. Third, completely revised and augmented edition. (Technique of Organic Chemistry, Vol. 1, Part 4). Pp. xii+2635–3539+65. 195*s*. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.)

THE general features of *Physical Methods of Organic Chemistry* have been indicated by the present reviewer when commenting on Parts 1 and 2, which were issued a year ago (*Nature*, **189**, 954, 1961). Parts 3 and 4, which have now appeared, keep to the same general type in that they are mainly written by physicists for chemists and do not explain sufficiently the scientific significance of the types of measurements which they describe. However, organic chemists to whom the volumes have, by title, been commended will find Parts 3 and 4 of much more interest than Parts 1 and 2, since these survey the major optical and electrical techniques in use to-day for the investigation of molecular structure.

Part 3 deals mainly with optical measurements. Two sections on "Spectroscopy and Spectrophotometry" from the old edition have now been expanded to five chapters without inclusion of superfluous material. The chapter on "Ultraviolet and Visible Spectroscopy" (150 pages) can be commended as a well-balanced review in which technical improvements are brought to notice and the structural significance of experimental results is explained in simple terms.

In contrast, the chapter on "Infrared Spectroscopy" deals almost exclusively with intrumentation and techniques, but this is to be excused since Volume 9 of the general series has dealt with the subject.

A number of following chapters on colorimetry, fluorescence, light scattering and polarimetry have few novel features, but, following these W. Klyne and A. C. Parker have written a new chapter on optical rotatory dispersion to deal with the major recent development of polarimetry which has had, in the past five years, a profound influence on knowledge of structural organic chemistry. Half this chapter is devoted to a judiciously selected survey of recent experimental data together with their cognate theoretical interpretations. The chapter can be commended as a concise, well-explained summary of this important subject.

Again, a new chapter by C. G. and R. J. W. le Fèvre on the Kerr effect is a useful addition to the treatise, but the same can scarcely be claimed of a later new chapter on the Faraday effect which, as yet, has found little practical or even theoretical applicability. Old, but slightly revised, chapters on determinations of dielectric constants and of dipole moments complete this volume.

Part 4 has several quite new chapters. Microwave spectroscopy is accorded 35 pages and, following this, nuclear magnetic resonance is surveyed by H. S. Gutowsky in a well-balanced 130-page chapter. The use of this important technique receives sufficient description for the chemist who wishes to be conversant with its applicability without having to delve too deeply into quantum theory. Following this the treatment of paramagnetic resonance, in 70 pages, is too heavily mathematical and fails to give an adequate impression of the value of this type of measurement. There is no indication that this type of measurement may be of particular value to biochemists in elucidating the behaviour of such complex substances as active preparations of enzymes.

After these chapters, that on the determination of magnetic susceptibility, by P. W. Selwood, is old and conventional. The author has discussed applicability in terms of organic chemistry but has failed to indicate the growing significance of this technique for inorganic chemists, although these scientists are more and more concerning themselves with organo-metallic compounds of diverse types.

Old chapters on conductometry, electrophoresis and polarography have been revised slightly and two new ones on determination of transport numbers and controlled-potential electrolysis have been added. The theoretical significance and the synthetic applicability of this last technique fully merit the attention which it has been accorded; these aspects of the subject receive adequate treatment. Determination of radioactivity has been accorded a hundred pages and is not over long, for there have been major developments of experimental techniques since the last edition of this book appeared. Very properly the chapter deals mainly with the counting of carbon-14 and of tritium, but describes, in full, the chemical procedures required for the conversion of organic substances into samples suitable for radioassay. Finally, the chapter on mass spectrometry, thoroughly modernized, closes the book. It again can be commended for devoting more than half its length to applications in both pure and applied organic chemistry. Due note has been taken of the conjoint use of mass spectrometry and gas chromatography.

The high cost of these volumes will undoubtedly restrict their sale, except to reference libraries, but, considering the excellence of the printing and the clarity of the diagrams, they could scarcely be produced more cheaply. However, since these two parts contain the chapters to which reference will most regularly be made, potential users should seriously consider the advisability of purchasing individual parts of the work rather than the complete set of four volumes. W. A. Waters

CHEMISTRY AND THE NERVOUS SYSTEM

The Neurochemistry of Nucleotides and Amino Acids

A Symposium of the Section on Neurochemistry, American Academy of Neurology. Edited by Roscoe O. Brady and Donald B. Tower. Pp. xii+292. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1960.) 80*s*. net.

Phosphorus Metabolism of Brain

By Dr. P. J. Heald. Pp. vii+195. (London and New York: Pergamon Press, 1960). 42*s*. net.

THE Neurochemistry of Nucleotides and Amino Acids, being a symposium of multiple authorship, exhibits considerable variety of style and approach among the thirteen main contributors. The articles on adenine nucleotides (by E. G. Trams), on guanine nucleotides (by D. R. Sanadi) and on the phosphatidic bases (by D. M. Greenberg) range far beyond the confines of neurochemistry. Dr. Greenberg's paper was originally delivered *in absentia*, and in its printed form is accompanied by a running commentary in footnotes from Dr. J. Stekol, who actually read the paper for Dr. Greenberg at the meeting on which this book is based. This novel variant of the 'invited discussion' may, as the sessional chairman observed, become a precedent for a new kind of symposium. Other notable contributions more directly oriented towards the nervous system are those by R. J. Rossiter and his co-workers on the cytidine nucleotides and by N. O. Kaplan on the pyridine nucleotides. The section on amino-acids contains a thoughtful review by H. Waelsch, with A. Lajtha, on protein metabolism in brain, and two papers on γ-aminobutyric acid which, however, deal mainly with metabolic minutiæ. Some of the recorded discussion might have benefited from rather more drastic editing, but Dr. Tower has written a useful summary which effectively rounds off this lively series of mainly neurochemical snippets.

Dr. Heald's book on the *Phosphorus Metabolism of Brain* is a slim volume with a high density of contained information, and, for a biochemical monograph, it has the distinction of not including a single chemical formula of the classical type. The opening pages summarize data on the composition of normal brain in respect of phosphorus compounds, the text being fully documented and embellished with several compendious tables. Such compression of material reflects much labour in compilation, but imposes some initial strain on the reader, although for reference purposes it enhances the value of the section. In later parts of the book emphasis shifts towards the functional correlates of the reactions described and the writing gains in fluency. Research

work based both on investigation of brains of whole animals and of isolated tissue preparations is considered, particular attention being given to the turnover of nucleotide and other 'high-energy' phosphates (where there is common ground between Dr. Heald and certain participants in the symposium reviewed above). The interpretation of experiments in which electric pulses are applied to slices of brain suspended in saline is judicially discussed, and a phosphoprotein which Dr. Heald has investigated figures in an intriguing, if speculative, scheme of reactions linking phosphate bond energy with ionic migrations in nerve cells. Other topics covered include the synthesis of phospholipids and the metabolic effects of anæsthetics; the book as a whole provides a comprehensive and timely survey of a field of study where general biochemical concepts such as that of energy-rich phosphate bonds may be related to the special features of a highly differentiated organ.

R. V. Coxon

FLUORINE CHEMISTRY

Advances in Fluorine Chemistry
Vol. 1. Edited by Prof. M. Stacey, Prof. J. C. Tatlow and Dr. A. G. Sharp. Pp. vii+203. (London: Butterworths Scientific Publications, 1960.) 45*s*.

Chemie der Organischen Fluorverbindungen
Von Milos Hudlicky. Pp. xviii+416. (Berlin: Veb Deutscher Verlag der Wissenschaften, 1960.) 51.60 D.M.

ADVANCES in Fluorine Chemistry is an excellent beginning at fulfilling a critical need in the field of fluorine chemistry, namely, a systematic presentation and appraisal of the chemistry embodied in the various major areas of this rapidly expanding field. To cover all the areas with the attention that is their due is a monumental task. The editors of this book have selected a number of prominent British research chemists and teachers to write on five areas. These are: the halogen fluorides (W. K. R. Musgrave), transition metal fluorides and their complexes (A. G. Sharpe), fluoroboric acids and their derivatives (D. W. A. Sharp), the electrochemical process for the synthesis of fluoro-organic compounds (J. Burdon and J. C. Tatlow), and exhaustive fluorinations of organic compounds with high-valency metallic fluorides (M. Stacey and J. C. Tatlow). Each chapter is written with every attempt made not to be redundant. Whenever an earlier book or review article has made a contribution to the particular area under discussion it is cited as a prime source. As a result, in general, more emphasis is placed on the reactions and fundamental nature of the chemistry of both inorganic and organic fluorides than on the general preparative procedures, for the various authors are aware that a systematic fluorine chemistry is developing, is ready for more than a casual interpretation in terms of the science of chemistry and that the time has arrived to do so in earnest. Although there is a minor tendency, in some parts of the text, to extrapolate a bit beyond what may be construed as conservative, the facts are presented thoroughly enough to allow the reader to make his own interpretations, if he chooses to do so. At the same time, some of the writers of this book are courageous enough to be mildly critical of the authors they are citing when the occasion demands it. This is not a common practice, but appears with sufficient frequency to be stimulating.

This book is no less than its title advertises. It should prove helpful to the fluorine chemist in keeping abreast with the five areas discussed. If detailed data on a compended variety of fluorine compounds is what is being sought by the reader, he is referred to the more than adequate bibliography that follows each chapter. Typical of the better books by British authors, this book is up to date (1959), well written and discriminating. On the lighter side, the careful reader is in for a mild surprise when he reaches p. 11, and finds that gravity acts differently in England and in the United States.

In the introduction, Prof. Stacey states that this is the first volume in a series, and that further volumes written by chemists from several countries are to be anticipated.

The German edition of the book by Hudlicky is essentially a translation of the original edition in Czech reportedly brought more up to date by the addition of material that appeared in the chemical literature between the dates of publication. The author admits that the book is the result of his own attempts to enlighten himself in the area of fluorine chemistry.

The book meets rather well the purpose for which it was published, namely, to present in broad aspect the materials, conditions, yields and methods for preparing organic fluorine compounds. To this extent, the work is well done. No attempt is made to cover every appropriate compound produced or to compile the properties of those that are reported. Reaction equations, with conditions and yields, are used liberally to account for the results reported. Each such equation has a bibliographical reference and all information is cross-indexed by paper, author and compound, making it very simple to find any desired preparation. Consequently, even a student with a minimal comprehension of scientific German can use this book to good advantage.

There has been no attempt made to write this book for any purpose other than as a suitable reference text for information *apropos* laboratory practice in preparing organic fluorine compounds. There are a number of short chapters on nomenclature, materials for handling fluorine, reactions of fluorine with various substance, hydrogen fluoride and inorganic fluorides. To complete the book, sections on fluorine analysis, freons, and plastics containing fluorine are considered briefly.

The bulk of the text involves methods for putting fluorine into organic compounds, the various preparations of organic fluorine compounds, and their chemical reactions. A short chapter on the general physical properties of these compounds completes the book.

There is no serious attempt to present a critical analysis of fluorine chemistry nor is any serious distinction made between organic fluorine compounds and fluorocarbon compounds. Most conclusions as to the mechanisms and nature of the chemistry cited are left to the discretion of the reader.

To sum up, this book is in no way an erudite but rather a practical reference book, that furnishes a helping hand to both the neophyte and the practising fluorine chemist to find methods for preparing, handling and using fluorine-containing materials. It could serve as a useful adjunct to the chemist's bookshelf.

Richard Dresdner

XUM

A Laboratory Handbook of Pulp and Paper Manufacture

By Dr. Julius Grant. Second edition. (Incorporating the fourth edition of Stevens's "Paper Mill Chemist".) Pp. vii+523. (London: Edward Arnold (Publishers), Ltd., 1961.) 80*s*. net.

PAPER is so common that one forgets that, in comparison with metals, plastics and other sheet materials, it has some very peculiar properties. It is a fibrous assemblage and frequently contains more air than solids; it may vary widely in density, strength and other physical characteristics, and it may be treated with a large range of chemical additives.

This handbook, like Stevens's before it, is written for laboratory workers with some previous knowledge of paper. It should, nevertheless, appeal to a wider range of readers because it effectively summarizes the enormous amount of work put in by chemists into one industry. It outlines the essential pulp and paper processes, then describes, in more detail, the laboratory methods developed for the control of these processes, the analysis of raw materials and the physical testing and analysis of paper. Many methods are only briefly summarized, as numerous references are given to published standard methods, and to the original literature, which is now very large.

It must be difficult to decide what to leave out, and in one sense it is a pity that the book is confined so closely to practical laboratory methods. There is little reference to theory, to the large amount of published work on the visco-elastic properties of paper, or to the development of instruments for automatic control. There are some who believe that chemists have had their day in the paper industry and that the future lies with technologists trained in physics and engineering. Actually co-operation between all three will be essential, but a common ground of theory is vital to this. Even though present theory is incomplete it is a big help in the understanding of the properties of paper and of specifications for it.

There are three misprints in the brief section on statistical method; they would not upset a statistician but might confuse a laboratory worker unfamiliar with that subject. F. Lyth Hudson

Papers on Bacterial Genetics

Selected by Edward A. Adelberg. Pp. xlvi+400. (Boston, Mass.: Little, Brown and Company, Inc., 1960.) 4.50 dollars.

Papers on Bacterial Viruses

Selected by Gunther S. Stent. Pp. xxx+365. (Boston, Mass.: Little, Brown and Company, Inc., 1960.) 4.50 dollars.

THESE companion paper-back volumes are worthy successors to the collection, *Papers in Microbial Genetics; Bacteria and Bacterial Viruses*, selected by Joshua Lederberg in 1951. Yet these two supplementing volumes only reflect a portion of the enormous activity devoted to this field in the decade since the first collection. Of the 51 papers now presented, only five have been retained from the former collection.

To abate the disappointment of many readers who, no doubt, would have selected differently in some instances, each collection is preceded by the selector's introduction, which reviews the main papers on each theme and includes a wide bibliography: on viruses, 13-page review, 164 references; on bacterial genetics, 30-page review, 177 references.

The papers themselves are presented in their original type and style, from which a certain lack of uniformity results; there is dual pagination; but most clearly brought home by this method of presentation is the need for a standard method of reporting references.

The great benefit of this collection is that it provides for the student of microbial genetics a highly concentrated source of information on both the methods and the results of the main workers in this field. Also, the combination of the bibliography in the introduction and at the end of each paper provides a fairly comprehensive guide to the other sources of related information.

To the specialist, therefore, these two volumes are almost 'essential'. L. B. Quesnel

A Laboratory Manual of Analytical Methods of Protein Chemistry

(Including Polypeptides). Edited by P. Alexander and R. J. Block. Vol. 1: The Separation and Isolation of Proteins. Pp. viii+254. (London and New York: Pergamon Press, 1960.) 50*s*. net.

THIS book is the first of three volumes devoted to practical techniques in the protein field, and contains seven chapters describing various methods for the separation and isolation of proteins. The first chapter (by Keller and Block) surveys the classical methods, such as precipitation with salts and organic solvents, for the separation of proteins. In chapter 2, Okunuki gives detailed descriptions of the methods, due mainly to Japanese workers, for the isolation of cytochromes, and bacterial α-amylase and proteinase. Chapter 3 comprises two sections. The first, by Keller and Block, reviews the techniques of adsorption and ion-exchange chromatography as applied to proteins, while the second, by Peterson and Sober, describes an ingenious variable-gradient device for column chromatography. In the next two chapters, Craig gives a full account of the use of dialysis and partition methods. The book ends with descriptions of the less commonly used techniques of multi-membrane decantation (Polson and Largier) and zonal density gradient electrophoresis (Svensson). It is to be hoped that the value of these methods will now be more widely recognized. Those readers who are not afraid of elementary mathematics will find that the theoretical bases of the various techniques are presented in a manner which does not engulf the practical aspects. A liberal number of plates and diagrams will enable workers in the less-affluent laboratories to construct much of the equipment described. In spite of a number of errors and a rather inadequate subject index, I feel that this volume will be extremely valuable to workers in the field of protein research. D. T. Elmore

An Introduction to the Chemistry of Heterocyclic Compounds

By R. M. Acheson. Pp. xiv+342. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 5 dollars; 35*s*.

THIS book has the laudable aim of supplying the student with a concise account of the properties and reactions of the more fundamental heterocyclic systems. In this it succeeds admirably. It covers the range from 3- to 6-membered heterocyclic compounds—with oxygen, sulphur, and nitrogen

as the hetero-atoms taken singly and (for the larger rings) in pairs—together with their important benzo-derivatives and the fused systems of purines and pteridines. Naturally occurring compounds of relevant interest are discussed briefly or mentioned incidentally, but carbohydrates and alkaloids are excluded. Although the scope is therefore wide, the main theme is the inherent chemistry of the commoner heterocyclic compounds. This is treated in sufficient detail to allow comparisons to be made both within and between the various classes of compound. The presentation is clear and the discussion modern, being supported by pertinent physical data and based on mechanistic principles. Moreover, as befits an introduction, the reader is given adequate guidance to further sources of information.

Such books as this must surely put an end to those perfunctory postscripts with which exhausted writers of text-books usually dispose of the subject. If ultimately this is service enough, more immediately the service to students and teachers is immeasurable.

Theory of Markov Processes

By E. B. Dynkin. Translated from the Russian by D. E. Brown. Edited by T. Kóváry. Pp. ix+210. (London and New York: Pergamon Press, 1960.) 60s. net.

THIS important and highly original work is devoted to a study of the foundations of the theory of Markov processes. It is written for the professional probabilist, and has little to offer to the non-expert, although any mathematician concerned with the teaching of measure theory will find it worth his while reading Chapter 1, in which the classical material is set out in a new and highly elegant form. This chapter also contains a careful account of conditional probabilities, some information about the new concept of "topological measurable spaces", and the best extension yet of the Daniell–Kolmogorov theorem on the construction of probability measures on function-space. The remainder of the book presents the first thorough formulation of the theory of Markov processes, starting with a whole collection of measures corresponding to all possible initial instants and all possible initial states, and allowing (as is now found convenient) the termination of the process at a random instant. The difficult theory around the concept of a 'strict' Markov process is set out in great detail. The present work is based on lectures given by the author in Moscow and Peking; it will be followed by a further volume devoted to the infinitesimal generators associated with Markov processes. It is very much to be hoped that the publishers will find it possible to translate this also.

D. G. KENDALL

Combinatorial Analysis

Proceedings of Symposia in Applied Mathematics, Vol. 10. Edited by Richard Bellman and Marshall Hall, Jr. Pp. vi+311. (Providence, R. I.: American Mathematical Society, 1960.) 7.70 dollars.

THIS volume records the proceedings of the symposium held at Columbia University during April 24–26, 1958. It consists of twenty-four papers and covers a very wide range of topics, including finite geometry, algebra, and number theory in pure mathematics; and statistics, communication theory and transportation in the applied field. A fairly recent development has been the use of an electronic computer to investigate combinatorial problems, and several of the papers describe results obtained. Some of these depend mainly on algorithms and others on somewhat complicated search techniques.

The papers on finite geometries include one by R. H. Bruck on "Quadratic Extensions of Cyclic Planes", one by D. R. Hughes "On Homomorphisms of Projective Planes" and one by A. A. Albert on "Finite Division Algebras and Finite Planes". Those on number theory include a paper by A. L. Whiteman on "The Cyclotomic Numbers of Order Ten" and the paper by O. Taussky and J. Todd on "Some Discrete Variable Computations". Thus pure mathematics is well represented in current activity in combinatorial analysis. But perhaps the main motive for this activity has come from operations research. Thus there is a paper by R. E. Gomory on "Solving Linear Programming Problems in Integers", one by M. Gerstenhaber on "Solution of Large Scale Transportation Problems", and a paper by M. M. Flood on "An Alternative Proof of a Theorem of König as an Algorithm for the Hitchcock Distribution Problem". There is also a paper by R. Bellman on "Combinatorial Processes and Dynamic Programming", and a paper by A. J. Hoffman on "Some Recent Applications of the Theory of Linear Inequalities to Extremal Combinatorial Analysis". These titles are sufficient to demonstrate the dynamic character of present-day research in combinatorial analysis. Anyone who moves in this field will wish to consult the proceedings.

L. S. GODDARD

Ciba Foundation Symposium on Quinones in Electron Transport

Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Pp. xii+453. (London: J. and A. Churchill, Ltd., 1961.) 60s. net.

THIS latest addition to the CIBA Foundation Symposia provides a most welcome review of recent work on the role of quinones in biological systems. As in previous volumes of this series, lectures by recognized authorities in the field are reproduced in full, the charts and diagrams often relating to hitherto unpublished material. Such treatment provides the atmosphere of the seminar rather than the formal lecture room, and the interest is further enhanced by the inclusion of the full discussion (diagrams again reproduced).

A detailed critique of a symposium volume of this type is quite out of place, but biochemists and organic chemists interested in the processes of biological redox systems will find stimulation and many useful references in this work. The symposium consists of the following lectures: isolation chemistry of ubiquinone (R. A. Morton, E. L. Crane, O. Isler and K. Folkers), coenzyme A and electron transport (D. E. Green); oxidation of quinones by mitochondrial preparations (E. C. Slater); oxidative phosphorylation (V. M. Clark, Sir Alexander Todd and P. J. Russell); biosynthesis of quinones (H. Rudney, A. J. Birch, O. Wiss and R. E. Olsen); biosynthesis of terpenoid side chains of quinones (F. Lynan); diet-induced changes in ubiquinone-levels (J. Green); function of vitamin K (C. Martius); spectroscopic observations of the redox reaction of ubiquinone in heart and kidney mitochondria (B. Chance); role of ubiquinone in the respiratory chain (E. R. Redfearn); histochemical studies (L. W. Wattenberg); role of plastoquinone in the electron transport system of photosynthesis (N. I. Bishop).

A. I. SCOTT

XUM

NATURAL RADIOACTIVITY IN TOBACCO AND TOBACCO SMOKE

By Dr. V. C. RUNECKLES

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FROM time to time reports on the levels of radioactivity in tobacco and tobacco smoke have appeared in both the lay and scientific Press. Mulvaney[1], Spiers and Passey[2] and Turner and Radley[3] have reported experimental work on the levels of radioactivity in cigarette smoke, while Ash[4] has calculated figures for the levels of activity in cigarette main-stream smoke based on its potassium content and the natural abundance of the radioactive isotope of potassium (potassium-40).

Interest in the level of natural radioactivity in smoke has come from two directions. First, the suggestion has been made[1] that the natural activity of cigarette smoke, resulting mainly from its potassium-40 content, is a cause of lung cancer. Secondly, interest in smoke activity has been aroused by Ash's claims[4,5] that the level of activity in cigarette smoke is so high that the cigarette smoke can be defined as a radioactive material since, according to his calculations, its specific activity exceeds a level of 2,000 μμc./gm., the basis of the definition according to the British Factories Act[6] and the International Atomic Energy Agency[7].

It therefore seemed desirable to obtain experimental information on the level of natural radioactivity in cigarette tobacco, ash and main-stream smoke, and to compare the observed level in smoke with figures obtained by calculation from its potassium content. Accordingly, plain cigarettes of both Canadian and American manufacture (74 mm. and 70 mm., respectively) were smoked to a butt-length of 23 mm. on a constant-pressure smoking machine, taking a 35-ml. puff of 2-sec. duration at a frequency of one puff per min. Main-stream smoke was collected in a train of three water traps followed by a 'Cambridge' filter. Cigarettes were smoked in batches of fifteen. The 'Cambridge' filter was replaced after smoking two batches. The cigarettes were extinguished with carbon dioxide. A total of 300 cigarettes was smoked in each experiment.

The contents of the traps were combined with washings of water and methylene chloride (to dissolve organic materials), evaporated to dryness in 'Vycor' crucibles and ashed at 450° C. for 18 hr. The residues after ashing were boiled in aqua regia until almost entirely dissolved. The samples were then taken to dryness, dissolved in 5 *N* hydrochloric acid, and aliquots plated on stainless steel planchets for counting.

The 'Cambridge' filters were ashed for 18 hr. at 450° C. and extracted with hot concentrated hydrochloric acid. The extracts were evaporated to dryness, the residues dissolved in 5 *N* hydrochloric acid and plated as above.

In order to determine the amounts of radioactivity present in the cigarettes before smoking, fifteen cigarettes comparable to those smoked were cut to 51 mm. (Canadian) and 47 mm. (American) and exhaustively extracted with water for 5–6 days (Soxhlet). The extracts were dried, ashed and treated as above. Ash collected from the cigarettes smoked in these experiments was digested with aqua regia until almost entirely in solution, dried and treated as already described.

The amounts of activity in the butts were determined as described for the unsmoked cigarettes. The activity present in a 23-mm. length of cigarette before smoking was calculated by proportionality from the values found for the unsmoked cigarettes. Subtraction from the observed activities of the butts led to values for the amounts of smoke activity trapped within the butts.

Measurement of radioactivity was made with a Baird–Atomic model *A2-2-27PF* proportional flow automatic counting system using a model 822 detector tube with a 'Mylar' end window and 90 per cent argon–10 per cent methane counter gas.

Because of the low count-rates obtained from the aliquots, and in order to minimize counting errors due to fluctuations in background count-rate, sample and background measurements were alternated, each being counted for the duration 900 counts, and each determination being repeated in sequence 50 times, that is, until 45,000 counts had accumulated per planchet.

The efficiency of the counter for potassium-40 was determined by measuring the activities of weighed samples of potassium chloride (analytical grade). A sample of potassium chloride was dried *in vacuo* over phosphorus pentoxide. Approximately 20 gm. were weighed accurately and dissolved in 100 ml. water containing 0·5 per cent gum arabic. Replicate planchets were prepared by accurately pipetting 200 μl. quantities on to stainless steel planchets, which were dried beneath an infra-red lamp. Half the planchets were placed in a furnace at 450° C. for 18 hr. After heating, all planchets were counted until 14,400 counts had accumulated for each. The mean count-rates for the heated and unheated samples were 7·9 and 7·7 c.p.m. respectively, showing that no losses occurred during heating. The mean sample weight of potassium chloride was 42·58 mgm. and the mean count-rate 7·77 c.p.m. The calculated potassium-40 activity (see below) was 36·1 d.p.m. leading to a counter efficiency value of 21·5 per cent.

The experimental results obtained in this work are presented in Table 1, which shows that almost all the activity present in the unsmoked cigarette can be accounted for in the main-stream smoke, ash and butt. Furthermore, approximately 90 per cent of it

Table 1. Natural Radioactivity in Cigarettes, Ash and Main-stream Smoke per Cigarette

Sample	Smoke B	Cigarette* A	Ash C	Trapped in butt D	Per cent recovery of activity $\left[\frac{B+C+D}{A}\right]\%$	Per cent transfer to smoke $\left[\frac{B}{A}\right]\%$
		μμc.				
1. Canadian 74 mm.	0·094	22·95	20·59	1·56	97·1	0·41
2. Canadian 74 mm.	0·084	—	—	—	—	—
3. American 70 mm.	0·159	21·20	17·90	0·52	88·8	0·75

* Activity of length of cigarette smoked to 23 mm. butt.

appears to remain in the ash, while less than 1 per cent is transmitted to the main-stream smoke. This finding is in agreement with the work of Spiers and Passey[2], who were unable to detect any activity in smoke, and concluded that the activity in the unsmoked cigarette remained essentially in the ash.

As pointed out above, each sample was counted until at least 45,000 counts had accumulated. For this number of counts the standard deviation is less than 0·5 per cent. However, considerably larger errors are associated with the various stages of sample preparation. The largest of these is undoubtedly associated with differences in sample size and thickness, giving rise to differences in sample geometry and self-absorption. While every effort was made to obtain uniform planchets, the pipetting of aliquots of the hydrochloric acid solutions and slurries led to uneven distribution of material.

The data for smoke activity are probably more accurate than those for cigarettes, butts and ash, since in this case sample preparation was more reproducible. However, the actual magnitude of the errors associated with these observations cannot be determined, but it would seem unlikely to exceed 10–15 per cent. In the light of this uncertainty, it is of interest to compare the observed activities for smoke with the value obtained by calculation.

Cogbill and Hobbs[8] found that potassium is the major metallic constituent of main-stream smoke, accounting for 135 μgm. of the 150 μgm. of metals in the smoke of one cigarette. The natural abundance of potassium-40 is 0·0118 per cent[9], and its half-life $1{\cdot}26 \times 10^9$ years[9], from which a simple calculation leads to a value of 0·113 μμc. as the potassium-40 activity in the main-stream smoke of one cigarette.

The figures for smoke in Table 1 are in general agreement with this calculated value. However, those for the Canadian cigarettes tested are somewhat lower, while that for American cigarettes is higher than 0·113 μμc. The American cigarettes smoked in this work and those used by Cogbill and Hobbs were blends of Burley and American flue-cured tobacco, while the Canadian cigarettes studied consisted exclusively of Canadian flue-cured tobacco. Since American flue-cured tobacco has a considerably higher potassium content than that grown in Canada, the difference in the level of smoke activity is perhaps not surprising.

If the 10–15 per cent figure suggested is a reasonable estimate of the error associated with these observations, it becomes clear that the measure of agreement between observation and theory suggests that the activity detected in main-stream smoke is due almost entirely to potassium-40, rather than to other isotopes, either natural or artificial. As Cogbill and Hobbs[8] point out, it is not surprising that potassium is the major metallic smoke constituent because it is one of the predominant mineral constituents of tobacco, and, as the chloride, possesses a distinct, though low, vapour pressure at the temperature of the combustion zone.

Table 2 lists the specific activities of the cigarettes studied and their main-stream smoke. In the case of the whole cigarettes the values are expressed as μμc./gm. of dry cigarette (tobacco plus paper), while in the case of smoke, the experimental values are expressed in terms of 'whole smoke'. These 'whole smoke' values were determined from the increase in weight of two 'Cambridge' filters in series when 15 cigarettes were smoked under the conditions described above, without the insertion of the water traps.

Table 2. SPECIFIC ACTIVITY OF CIGARETTES AND MAIN-STREAM SMOKE

Sample	Cigarette			Smoke		
	Activity μμc.	Wt. smoked gm.	Specific activity μμc./gm.	Activity per cig. μμc.	Wt./cig. gm.	Specific activity μμc./gm.
1. Canadian	22·95	0·675	34·0	0·094	0·026	3·62
2. Canadian	—	—	—	0·084	0·026	3·23
3. American	21·20	0·592	35·8	0·159	0·029	5·49
4. American	—	—	—	0·113*	0·072†	1·57

* Calculated from data of Cogbill and Hobbs (ref. 8).
† Reported by Cogbill and Hobbs.

The values for the specific activity of smoke are of primary interest in connexion with the suggestion that smoke can be defined as a radioactive substance[4]. Unfortunately, the figures on which Ash bases his claims are incorrect since they are based upon his calculated value of 0·01 mμc. of potassium-40 activity in the main-stream smoke of one cigarette. Thus when expressed in terms of 150 μgm. of smoke metals, the specific activity of smoke using the correct value of 0·113 μμc. per cigarette is 0·667 mμc./gm. Ash more recently[5] has used this value rather than the value of 6·67 mμc./gm. quoted in his original paper. However, all these calculations of specific activity are unrealistic since they are based upon the weight of metallic smoke constituents alone. A more reasonable basis for calculation is the weight of whole smoke, exclusive of gases, since the potassium-40 activity of smoke is distributed throughout its particulate matter as a whole. The results in Table 2 are calculated on this basis, and show clearly that the specific activities of the smoke from the cigarettes studied are several orders of magnitude less than the level of 2,000 μμc./gm.

Ash's view that smoke is definable as a radioactive substance[5] thus has its basis in the unrealistic use of smoke metals alone for calculating specific activity. However, his further statement that at 667 μμc./gm. the specific activity of smoke is only one-third of the level of the definition, but that this level should be reduced to one-tenth when applied to the population as a whole is without meaning. His point that it is generally agreed that the levels of radioactivity permitted for occupational exposure are ten times those permitted for the general public is irrelevant when dealing with specific activities; for permissible levels are based upon considerations of total accumulation of irradiation, irrespective of the specific activity of the source. As a consequence, the safety factor of one-tenth cannot meaningfully be applied to the 2,000 μμc./gm. level of the definition[6,7].

In connexion with the view that the radioactivity of tobacco smoke is the cause of lung cancer in the smoker, several questions can be raised. For example, how does the amount of activity inhaled by the smoker compare with his intake of natural activity in the diet? How does the inhaled activity compare with the equilibrium-level of activity in the body? How rapidly are mineral smoke constituents eliminated from the lung. How does the level of activity in the particulate phase of smoke compare with the natural level of radon and thoron activity in air?

Little information is available in the literature on the absolute levels of the naturally occurring isotopes, in contrast to those of the isotopes strontium-89, strontium-90 and cæsium-137 which have come into prominence recently as constituents of 'fall-out'. Table 3 presents a collection of results obtained from various sources. Where so indicated, the levels of

Table 3. NATURAL RADIOACTIVITY IN MAN'S ENVIRONMENT

Source	Activity	Ref.
Air	10^{-4} μμc./ml.	10
Air	3×10^{-5} μμc./ml.	12
Human body	120 mμc. ^{40}K, total	10
Sea water	0·33 μμc. ^{40}K/ml.	10
Drinking water	1 to 10^{-4} μμc./ml.	10
Apples	1·00 μμc./gm.*	11
Almonds	7·24 μμc./gm.*	11
Boiled cabbage	1·21 μμc./gm.*	11
Boiled spinach	4·10 μμc./gm.*	11
Dried apricots	16·10 μμc./gm.*	11
Wheat bran	10·50 μμc./gm.*	11
Cow's milk	0·01 μμc./ml.	10

* ^{40}K activity of the potassium contents of these foods, calculated from the data of ref. 11.

activity are for potassium-40 alone, and are calculated from potassium content. A comparison of the results from Tables 2 and 3 shows that the specific activity of the unsmoked cigarette is higher than the specific activity of any of the foodstuffs listed, which were selected to cover a wide range of potassium contents. This difference is, however, to be expected, since tobacco has a relatively higher potassium content than most food crops because of the fertilization practices used in its cultivation. In addition, the tobacco in the cigarette is in a partly desiccated form, and it can be seen that the level of its activity is approached by those of the two dry food materials listed, namely, dried apricots and wheat bran.

Relating the level of smoke activity to total uptake of activity by the body, it should be emphasized that all food materials contain natural radioactivity to some extent. The average dietary intake of activity is difficult to estimate since it depends so much upon the nature of the diet, geographical location and, more recently, upon the contamination of foodstuffs by 'fall-out'. Mayneord[12] has shown that approximately 20 per cent of the dose-rate received by the body arises from its potassium-40 content, while external sources such as cosmic radiation and local radiation from the ground and building materials provide approximately 70 per cent. Internal radiation from isotopes other than potassium-40, for example, carbon-14 and the radium, thorium and uranium series, probably gives rise to less than 5 per cent of the total body irradiation.

Since experimental evidence suggests that potassium is by far the most important isotope in smoke, and since this same isotope is the major source of internal radiation in the body, it is of interest to look at the average diet from the point of view of its potassium-40 content.

The figures in Table 4 are calculated from values for daily potassium intake in the United States published by Anderson *et al.*[14]. Of the daily uptake of approximately 3 gm. potassium (2,560 μμc. potassium-40), 2·6 gm. (2,180 μμc. potassium-40) are excreted daily in the urine, while the remainder is eliminated in the fæces and sweat. The rate of total

Table 4. DAILY POTASSIUM INTAKE, BASED ON *per capita* FOOD CONSUMPTION IN THE UNITED STATES

Source	Potassium (gm./day)	Potassium-40 (μμc./day)
Dairy products	1·15	960
Meats	0·46	390
Flour and cereals	0·20	170
Vegetables	0·56	470
Citrus fruits	0·10	80
Potatoes	0·59	490
Total	3·06	2,560

elimination is determined by the need to maintain an average bodily equilibrium level of approximately 133 gm. potassium (120 mμc. potassium 40) (ref. 12).

The calculated potassium-40 activity of cigarette smoke (0·113 μμc. per cigarette) is a very small fraction of the dietary intake, and, even in the case of the heavy smoker, smoking, say, 50 cigarettes a day, the intake of potassium-40 from smoke would be less than 0·25 per cent of the daily intake from food.

However, this extremely small amount of activity could be important if the potassium-40 remained in the lung for a long period, that is, if the lung accumulated mineral smoke constituents, including potassium, without elimination or absorption.

Table 5. FATE OF PARTICULATES IN RESPIRATORY TRACT IN STANDARD MAN

Distribution	Readily soluble compounds (per cent)	Other compounds (per cent)
Exhaled	25	25
Deposited in upper respiratory passages and ultimately swallowed	50	50
Deposited in lungs	25 (taken up in body fluids)	25 (12·5 per cent eliminated in first 24 hr.; remainder eliminated with a half-life of 120 days into body fluids)

Table 5 shows the fate of particulate matter in the respiratory tract as calculated for the standard man[13]. This indicates that, in general, soluble particulate matter is deposited in the lung to the extent of about 25 per cent and is then absorbed into the blood stream. Less-soluble materials are deposited to about the same extent, but about half of these are eliminated in one day while the remainder have a biological half-life of about 120 days. Since the potassium in smoke is likely to be in a readily soluble form, it would seem probable that it is eliminated quite rapidly. The work of Wolff and Purdom[13] suggests that this is so. They allowed dogs to inhale tobacco smoke containing various metal tracers and determined the level of activity of these tracers in the lung tissue at intervals after the smoking period. In the case of potassium-42 they found that nearly all activity disappeared from the lung during the smoking period or soon thereafter. Since potassium-40 and potassium-42 are chemically identical, these results suggest that potassium-40 taken into the lung during smoking is also rapidly eliminated from that organ.

These results combine, therefore, to suggest that cigarette smoking does not overburden the body from the point of view of irradiation by potassium-40, nor does it produce an appreciable increase in radiation to the tissue of the lung.

Relevant to this last point, it is of interest to compare the intake of potassium-40 activity from smoke with the natural activity of air, due to its content of radon and thoron. These gases decay with the production of heavy metal isotopes and in the process emit α-particles which are much more damaging to tissue than the β-particles of potassium-40. On the basis of 11 puffs of 35 ml. vol. in the case of the Canadian cigarettes smoked in this work, the specific activity of their main-stream smoke can be calculated as approximately $2{\cdot}4 \times 10^{-4}$ μμc./ml. In the case of the American cigarettes,

approximately 9 puffs were taken per cigarette, leading to a figure of about 5×10^{-4} μμc./ml. The levels of radon-thoron activity in air are shown in Table 3. Using Mayneord's value of 3×10^{-4} μμc./ml., it is apparent that the levels of potassium-40 activity in smoke are of the same order of magnitude.

Further to this, Turner and Radley[3] have studied the α-particle activity of tobacco, due to its radium and thorium contents, and have concluded that the release of α-activity during smoking is extremely small. They state that even in the case of the heavy smoker, the additional α-activity taken into the lungs is less than 1 per cent of the atmospheric radon and thoron inhaled daily by any individual, approximately 2 mμc., a figure obtained from the approximate daily uptake of air, 2×10^7 ml. (ref. 15) and its radon-thoron content, 3×10^{-4} μμc./ml. (ref. 12).

In summary, therefore, we can say that the observed activity of cigarette main-stream smoke appears to be due almost entirely to its content of potassium-40. The amounts of this activity taken into the lung, even by the heavy smoker, are minute compared with the bodily uptake from the diet, and are rapidly eliminated from the lung tissue. In addition, this potassium-40 activity only exceeds by a small amount the α-activity taken into the lungs of all individuals because of the radon and thoron in the Earth's atmosphere.

Finally, calculations of the specific activity of smoke, based upon a logical appreciation of its nature, lead to values far below the internationally agreed level at which any material can be defined as radioactive.

I wish to thank the Directors of the Imperial Tobacco Co. of Canada, Ltd., for permission to publish this article, and to acknowledge the assistance of Mr. B. J. Stirling and Mr. K. Woolrich in the experimental work.

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CLIMATE AND WEATHERED KARROO DOLERITES

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Introduction

A VERY important road-building material in large parts of the Union of South Africa is Karroo dolerite, the weathered rock being used in the layers of road foundations and the fresh rock when crushed for surfacing chips. The performance of weathered dolerites is known to vary in different parts of the country. The material performs well in the western portion of the Union and badly in the eastern, the differences in performance being borne out by observation that the rate of alteration of the materials, after being placed in the road foundation, appears to increase. These facts led me to carry out research on the influences of environmental conditions on the alteration of basic igneous rocks with particular reference to Karroo dolerites.

The most conspicuous feature of the South African Karroo system is the huge mass of crystalline igneous rock which is called traditionally 'dolerite'; according to present nomenclature, however, it should be designated as 'diabase' of a very wide variety[1]. These dolerites are intrusive into the sediments of the Karroo era in numerous thin dykes and in sills up to a thickness of 200 m. or more. There is a marked southern limit to the extent of these rocks, crossing the southern portion of the Karroo areas in a roughly west–easterly direction (Fig. 1).

The rocks are dark-coloured generally, varying due to structure, texture and mineral composition from norite to quartz-diorite, with diabase forming the bulk of the whole occurrence. Differentiation may lead to light-coloured rocks exceptionally.

Weathering of Dolerites

Dolerite outcrops in the eastern parts of the Union of South Africa show a succession of weathering products consisting normally of four layers which can be distinguished readily by their colour and consistency. The lowest layer is the fresh rock or that which, in comparison with the rest, may be termed

Fig. 1. Occurrence of the Karroo system in South Africa

'fresh'. This rock is dark in colour with the whitish feldspars standing out clearly in the brownish- to greenish-black matrix which consists of pyroxene, amphibole, different ores and occasionally olivine, biotite and quartz. The rock is hard and dense, giving a metallic ring when struck with the hammer. The next layer, the weathered rock, is still hard but brittle, breaking readily when struck with the hammer, and often showing exfoliation. The colour is brownish due to frequent staining with iron oxides. The feldspars are still clearly recognizable, while most of the mafic minerals have lost their dark colour. The third layer, the badly weathered rock, has lost its consistency completely and become a brittle to soft, yellowish-grey mass with an earthy feeling. The original structure of a crystalline igneous rock is still preserved, and the former planes of exfoliation are clearly visible: most of the original minerals, however, have lost their properties completely. The fourth layer is the residual soil on the top of such deposits. These soils vary, depending on climate and relief, being black clays in water-logged areas in most cases and deep red clays on slopes[2].

The weathering succession in dolerite outcrops in the western portions of the country differs somewhat from the profile given in the previous paragraph. The stages 'badly weathered' and 'residual soil' are not properly reached and the whole succession remains in the state 'weathered', as defined. However, the rock does not remain a hard material but merely disintegrates, finally becoming a dolerite gravel, the grains of which are the different minerals of the original rock, showing only partial chemical alteration. Physical weathering is clearly overshadowing chemical weathering in the western portions of the Union, this statement being in some contrast to others made previously[3].

An increasing amount of sand normally occurs towards the surface, being blown in, and consequently mixed with, the residual soil. The difference between clays, black or red, in the eastern parts of the country and sandy to gravelly soils with only little clay, is also expressed by the predominance of the soil clay minerals in the widest sense. Hydrous mica is predominant in the west, and other clay minerals, montmorillonite and kaolin in particular, in the east[4].

A statistical investigation on the percentage of secondary minerals in the different layers of the weathering successions has been carried out for a number of samples from different sites. Similar investigations had been made previously on certain basalts in Oregon, United States[5]; the South African approach is different from the American one in so far as the latter dealt with rocks from rather uniform environments in regard to topography and climate, while the South African investigation has covered materials from many different regions.

Quartz, feldspar, biotite, amphibole, pyroxene, olivine and magnetite were considered primary minerals and all others as secondary. The investigation was carried out with a polarizing microscope and counting was done with the aid of a micrometer. Special difficulties were experienced during the preparation of the badly weathered parts and the residual soils of the weathering succession. For grinding purposes, resins had to be used in order to harden these materials. All minerals which could be recognized individually were counted separately, and those too small for recognition under the microscope, such as clay minerals, were just counted as 'secondary'. The results of counting were arranged in two groups, primary and secondary minerals, and the relation was expressed as a percentage of the total number counted.

The results were compared with the field description of the weathering succession as 'fresh', 'weathered', 'badly weathered' and 'residual soil', and a remarkably good correlation of these designations with certain ranges of percentages of secondary minerals was observed:

Field description of rock	Percentage of secondary minerals
Fresh dolerite	< 25
Weathered dolerite	25–32
Badly weathered dolerite	> 32
Soil, residual from dolerite	> 70

The small range, 25–32 per cent, for the section 'weathered dolerite' appears to be the most remarkable result of this investigation, because it shows that the transition from the solid, fresh-looking rock to the thoroughly decomposed material seems to be a rather abrupt change. The number of samples studied so far has not allowed a range of percentage to be allocated to the group 'badly weathered'; the limit between 'badly weathered' and 'residual soil', however, may be expected somewhere between 60 and 70 per cent.

X-ray investigations have shown that montmorillonite and hydrous mica occurred in all samples, including those which were classified as 'fresh'. Montmorillonite is well crystallized in these samples and also in some of the 'weathered' and 'badly weathered' ones; it never appeared crystallized in the residual soil, but only as amorphous material. The transition from the crystallized to the amorphous states appeared to take place in the stages 'weathered' and 'badly weathered'. Kaolin may occur suddenly at a certain level in the 'badly weathered' stage, being then associated with an almost complete disappearance of all feldspars.

The results of the work on secondary minerals, as well as from X-ray and differential thermal analysis investigations, were the same for samples from the western and the eastern portions of the country as regards the classification from 'fresh' to 'residual soil'. In the western part of the country, however, the state 'weathered' was not passed.

Standard engineering tests carried out on those weathered dolerites which were intended for road construction indicated a regional distribution of the results. This was the case for the Atterberg limits in particular, where the liquid limit and the plasticity index were of special significance and importance. A line could be drawn across the Union with the values of 30 for the liquid limit and 10 for the plasticity index, larger values predominating to the east and lower values to the west of this line. This line intersected the coast line near Port Alfred and crossed the Orange Free State a little east of Bloemfontein; it then continued towards Rustenburg in the Transvaal, following thus a generally north–south course. This also agreed roughly with the types of weathering given above, disintegration prevailing on the western and decomposition on the eastern side of it.

Climatic Investigations

The Union of South Africa falls within three major climatic regions. They are the summer rainfall areas which cover the largest parts of the country, the winter rainfall areas of the Cape of Good Hope and

the almost constantly dry desert regions of the north-west. There are remarkable variations within the large summer rainfall areas, concerning the duration of the rainy season and the quantity of water precipitated. The climatic investigations were concentrated on the summer rainfall areas because only those areas are bisected by the line which separated weathered doleritic materials on the basis of the Atterberg limits and which also agreed with the boundary between the types of weathering.

The Orange Free State was chosen for the first detailed investigation for three reasons: (1) the Orange Free State falls completely within the summer rainfall area; (2) the province is relatively small and in a central position within the country; (3) weathered dolerites are almost the only road-building materials within the province and a sufficient number of test results was available.

The climatic investigation included precipitation, potential evaporation calculated for a shallow free-water surface from the Meyer formula[6], relative humidity, wind speeds, temperature and a calculated ratio of evaporation to precipitation. All investigations were made for the months of January (rainy season) and July (dry season) separately, giving thus separate pictures for the climatically extreme parts of the year. The annual precipitation and a ratio of the relative humidities of January to those of July were also added.

The evaporation precipitation ratios were calculated so that the higher value was always divided by the lower one, giving results representing either a surplus of evaporation over precipitation (positive values) or the opposite (negative values); the numerical figures then are comparable. Contours were drawn on the basis of the values obtained, the contour 1·0 connecting places with equal evaporation and precipitation. The contour +3·0 for January appeared to be the most significant in comparison with the lithological boundaries.

Contours were drawn for each climatic indicator, the general contour pattern being compared with the boundary obtained from the test results. Those contours which agreed with this boundary were selected. The most remarkable feature of the results was that, while none of the contours referring to July agreed with the soils boundary, agreement was found for the January values and, furthermore, that all climatic indicators showing an agreement had something to do with water. Besides these monthly values, a close agreement occurred with the annual isohyet of 500 mm. and with a contour connecting all those points where July has the same relative humidity as January. This contour runs a little west of the zone occupied by the other contours. It appears to be of importance, as the Orange Free State falls completely within the summer rainfall area, where January would be expected to be more humid than July. However, the relative humidity of July is higher than that of January in the country west of this line. This fact indicated that the inflow of moist air in January is not high enough to compensate for the higher temperatures in this month.

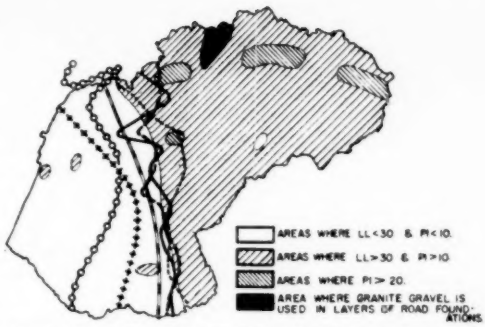


Fig. 2. Climatic and soils boundaries in the Orange Free State. – – –, Relative humidity of 55 per cent in January; + +, relative humidity in January = relative humidity in July; ——, 85 mm. isohyet for January; — —, evaporation/precipitation ratio + 3·0 in January; ○, boundary between hydrous mica (west of line) and other clay minerals (east) as predominant soil clay minerals; ■□■, 500 mm. isohyet of annual precipitation; *LL*, liquid limit; *PI*, plasticity index

The border between the predominance of hydrous mica and other clay minerals in South African soils as indicated by C. R. van der Merwe and H. Heystek[4] was also added. This border runs somewhat west of that defined by the Atterberg limits, which may be one indication that a certain percentage of clay minerals, montmorillonite in particular, must be present in the unsound weathered dolerites to make them differ from the sounder materials of another region.

The position of all these contours, relative to the subdivision of the weathered dolerites in the Orange Free State based on the Atterberg limits, is shown in Fig. 2.

These investigations were then extended over the whole Union of South Africa and contours were drawn again. The same climatic values as for the Orange Free State were sorted out and it was noted that their meaning was the same as before in the eastern Cape Province and the western Transvaal, the whole of Natal being east of the critical boundary. All contours then enter the Bechuanaland Protectorate. From 25° S., however, the 500 mm. isohyet of annual precipitation, together with the boundary of hydrous mica as the predominating soil clay mineral, turns east and re-enters the Transvaal again, but the others retain their approximate northerly direction (Fig. 3). This course of the contours leaves the deeply weathered Bushveld norites—the depth

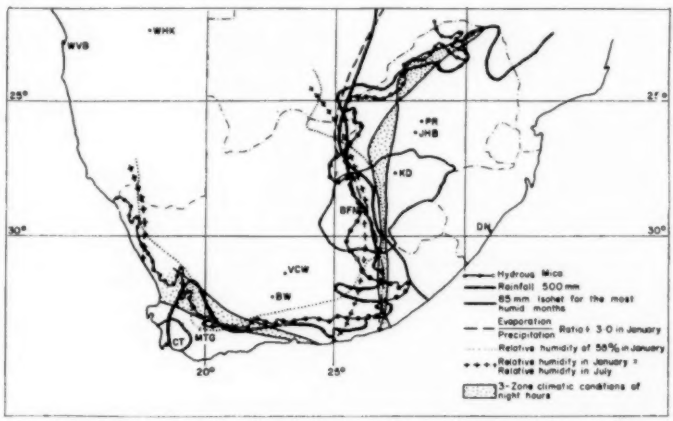


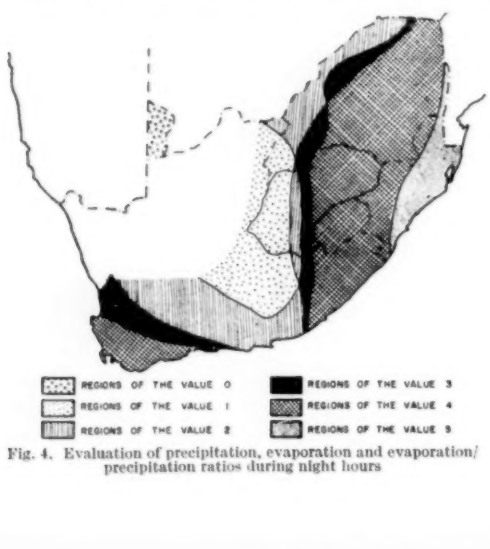
Fig. 3. Climatic and mineralogical features concerning weathering of Karroo dolerites

of weathering is known to be more than 8 m.—on the eastern side of the zone created by the climatic contours. Dolerites do not occur in this part of the Union, but the norites can, however, be considered sufficiently similar to them for such comparison.

Assuming that the effect of water on weathering will increase if evaporation is reduced considerably, a separate investigation on precipitation, evaporation and the ratio of evaporation to precipitation was carried out for the night hours of January, that is, the period 1900–0500 hr., and July, that is, the period 1700–0700 hr.

Contours were again drawn for precipitation, evaporation from a shallow free-water surface and the ratios of evaporation to precipitation. The contour patterns of these three indicators, separately drawn for January and July, were compared with the soils boundary as obtained from the Atterberg limits and it was found that all patterns for January agreed with this boundary, but there was no agreement with the July contour patterns. Those January contours which agreed best with the soil boundaries were selected and used as critical values for the climatic effects on weathering. These critical values were: (1) evaporation during night hours 50 mm., its effect on weathering being greater with decreasing values; (2) precipitation during night hours 30 mm., its effect on weathering being greater with increasing values; (3) evaporation/precipitation ratio during night hours of 1·0, that is, evaporation equal to precipitation. A greater influence on weathering would be expected where surplus of precipitation occurs.

In order to gain an overall picture, numerical values were allotted to all areas of the country on the base of the above subdivision. These were 0 for weak and 1 for strong effects on weathering of the individual climatic conditions during night hours[7]. This evaluation led to the subdivision of the country into six different zones, each one designated by one of the numerical values 0, 1, 2, 3, 4 and 5 (Fig. 4), which means that the higher the numerical value of a given area the stronger the potential influences of all climatic indicators. Two relatively narrow zones, one of them bisecting the summer rainfall area and the other bordering the winter rainfall area of the Cape, have the value 3. The 3-zone of the summer rainfall area agrees well with the full-day contours, as already known from the Orange Free State, as well as with the soils boundary defined from the Atterberg limits and from the predominance of either disintegration or decomposition for that portion of its course which passes the western Transvaal, the Orange Free State and the eastern Cape Province. This zone becomes ill-defined in its northern continuation through the Transvaal, where the easterly type of weathering is known to cross this zone near Rustenburg and Brits, and here the 2-zone seems to take the place of the 3-zone. No conclusion can be drawn about the relevance of the 2-zone in the winter rainfall area of the Cape, where the bulk of rain falling during the colder part of the year might have a somewhat different effect on the weathering of basic igneous rocks. It may be mentioned, however, that more intense weathering of dolerites is known to occur in the vicinity of Calvinia, which is near the 3-zone.


REGIONS OF THE VALUE 0
REGIONS OF THE VALUE 1
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REGIONS OF THE VALUE 4
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Fig. 4. Evaluation of precipitation, evaporation and evaporation/precipitation ratios during night hours

Conclusions

The comparison of the present climatic conditions of a country with very marked differences between a humid, warm and a dry, cool season, with the types of weathering and with some engineering properties of weathered basic igneous rocks, dolerites in particular, has emphasized clearly the importance of water. All climatic indicators which had an apparent bearing on weathering referred to the climatic conditions of the warmest and most humid month, and all of them were connected with the direct or indirect influences of precipitation or water. The close agreement of a number of climatic contours, demarcating regions with different types of weathering and, at the same time, with different engineering performances of the weathered materials, holds for the larger portion of the South African summer rainfall area. This climatic zone, however, becomes ill-defined towards the north, where increasing mean air temperatures as well as increasing soil temperatures might strengthen the influence of water, that is, a smaller amount of water at a warmer temperature may have the same effect on weathering and on engineering properties as a higher amount of water at lower temperatures. In the winter rainfall areas of the Cape Province no such close agreement of the various climatic contours occurred. A larger temperature effect may also appear here, because the rain falls during the colder part of the year.

Microscopic and X-ray investigations showed that the material alteration which the dolerites undergo during weathering is the same on either side of the climatic separation zone. The percentage of secondary minerals and the type and state of clay minerals were the same, independently of the local climate, if a certain state of weathering were reached.

The rate and progress of weathering, however, can be connected with the local climatic conditions. In the western parts of the country, that is, in the climatic zones 0 and 1 (Fig. 4), decomposition is reduced in favour of mere disintegration and does not normally pass the state 'weathered' of the climatic zones 4 and 5. In the eastern parts of the Union, that is, in the climatic zones 4 and 5, little disintegration occurs and the process of weathering is largely chemical in nature. The 2- and 3-zones are largely transition zones between the other two.

The differences in weathering also refer to the performance of the weathered materials if used for road construction and, especially for foundation purposes, with decomposition leading to unsound materials and

poor results, and disintegration leading to sound materials.

The climatic conditions of a given site or area, the amount of water available in particular, and probably also a combination of air and soil temperatures, may thus provide a means for defining the probable type of weathering and also for predicting the engineering behaviour of weathering or weathered basic igneous rocks, among which the Karroo dolerites are of great practical importance in South Africa.

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MAGNETIC STORMS IN INTERPLANETARY SPACE AS OBSERVED BY *PIONEER V*

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Space Technology Laboratories, Inc., Los Angeles, California

IN a preliminary report on the magnetometer experiment carried by the deep space probe *Pioneer V* (1960α), Coleman *et al.*[1] display a correspondence between fluctuations in average ambient magnetic field value at the probe and local a_k magnetic index values recorded concurrently at Fort Belvoir, Virginia, for the very active period March 30–April 3, 1960. Fig. 2 of their paper exhibits the satellite data and the a_k index plotted in parallel, showing agreement between the two traces for the intense magnetic perturbation of April 1 and for lesser events of March 31, April 1 and April 3. The present article extends the comparison between effects at the Earth and at *Pioneer V* over the entire period of regular acquisition of data in the interplanetary region, showing a correlation between 'storms' appearing at the two bodies, and between the interplanetary field and planetary magnetic index, a_p.

The trajectory of *Pioneer V* is shown in Fig. 1. Only field magnitude perpendicular to the axis of rotation of the spin-stabilized probe (arrows) was measured, and only relative changes in the measured component are considered here. Details of probe and magnetometer geometry have been published elsewhere[1,2].

Launched on March 11, 1960, the probe reached a distance of 500,000 km. by the middle of March 12, placing it well outside the terrestrial magnetic field[1,3,4]. The distance between the payload and the Earth increased continuously to approximately 12,000,000 km. on April 30, after which telemetry transmission became too infrequent for significant addition to the results.

Measurements were transmitted as several discrete messages each day, with each message averaging 30 min. duration and including many individual magnetic field readings. Individual readings were averaged over intervals of half a day for this work. The half-day interval has proved to be a convenient unit because it is long enough to diminish the influence of short-term transients, while not so long as to obscure the correlation between short magnetic storms. A smoothed graph of the data has been produced in turn by plotting, instead of each half-day field average $\bar{B}_i$, the mean of $\bar{B}_{i-1}$, $\bar{B}_i$, and $\bar{B}_{i+1}$. The upper curve of Fig. 2 presents this smoothed version of the average ambient field component at the payload, with concurrent geomagnetic storm periods and smoothed planetary indices below for comparison.

The magnetic index curve was prepared in the same manner as that used for payload data : the 3-hr. planetary index a_p, converted from published K_p values[5–7], was averaged over half-day intervals and a three-interval mean applied to the half-day averages.

Periods designated as separate geomagnetic storms by one or more reporting observatories[8,9] are represented in Fig. 2 by solid horizontal bars. Dashed bars appear in connexion with the March 14 event, where there is substantial disagreement on duration of the storm and later where some observatories reported single long events rather than separate occurrences.

Statistical correlation between payload field increase and separate geomagnetic storm incidence is particularly striking. The curve of smoothed payload

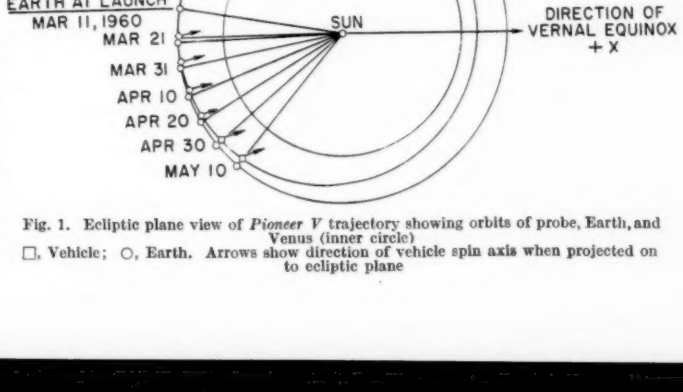

Fig. 1. Ecliptic plane view of *Pioneer V* trajectory showing orbits of probe, Earth, and Venus (inner circle)
□, Vehicle; ○, Earth. Arrows show direction of vehicle spin axis when projected on to ecliptic plane

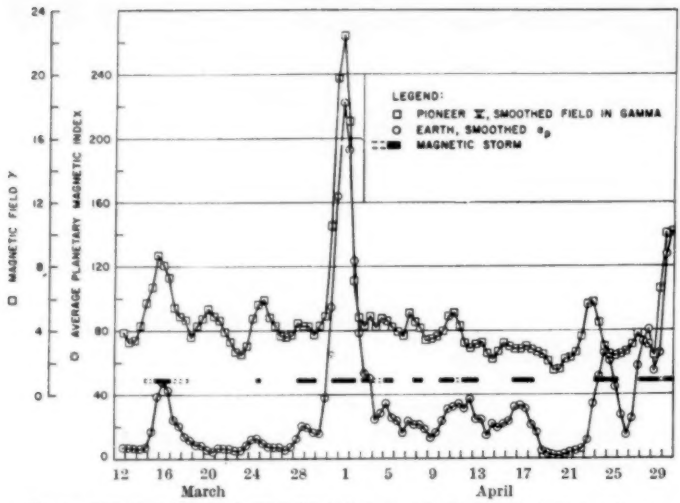

Fig. 2. Smoothed *Pioneer V* magnetic field, smoothed planetary index a_p, and geomagnetic storms

ambient field, characterized by a sequence of crests or excursions of diverse amplitude and more or less similar shape, has 14 maxima and a rapid rise before the graph terminates on April 30. Subsequent data, not shown, give a return to nominal field readings on May 1, so that a fifteenth peak may be added to those visible in the figure.

Of the 15 maxima in the satellite data, 14 are accompanied closely or followed within one day by the occurrence of magnetic storms at the Earth. Of the total of 13 individually reported geomagnetic storms occurring during the fifty days of observation (solid bars) every one is accompanied or preceded within a day by an excursion of the smoothed ambient field at the payload. The geomagnetic storm in the interval April 16–18 can be associated with a pair of local submaxima in the smoothed payload data, making as before a total of fourteen correlated peaks at the probe. The one exception to the agreement between probe data and storm reports is the excursion of March 19–21, but examination of magnetic records covering this interval shows that while no magnetic storm appeared in the reference list, disturbances in the Earth's field, noted particularly on College, Alaska, magnetograms, appear to have taken place (see discussion of magnetic index following).

The plot of smoothed planetary index in Fig. 2 consists of a series of irregular excursions comparable to those appearing in the *Pioneer V* curve. Qualitative agreement between the two measurements can be seen at once, with the extreme maxima of April 1 and 30 as outstanding parallel features. Some details of the March 30–April 3 period have been lost by smoothing, but evidence of correlation for separate peaks of April 1 and 3 can be found in the preliminary report mentioned earlier[1]. Once again, the payload excursion of March 19–21 appears uncorroborated at the Earth, despite the faint trace of corresponding activity in the planetary index. Indication of high-latitude terrestrial effects on March 19 and 21 is present, however, in magnetic indices from College. This can be seen in a comparison of the payload daily average field and College A_k index, reproduced in Fig. 3 for the interval March 12–28.

The data discussed here and presented in Figs. 2 and 3 show that relative increases in the average value of field amplitude measured by *Pioneer V* almost always occurred in approximate conjunction with magnetic storms at the Earth's surface and consistently with increases in irregularity of the Earth's field as measured by the magnetic indices a_p and A_k. It is tentatively concluded from this evidence that enhancement of at least one component of nearby interplanetary magnetic field regularly accompanies terrestrial magnetic storms. It is also concluded that this enhancement cannot be restricted exclusively to a component directed along radial lines from the Sun, since during an appreciable part of the observation period the probe magnetometer measured field magnitude in a plane nearly normal to the Sun-payload radius (normal to the arrows of Fig. 1).

A feature essential to the correlations between payload and planetary index curves and between payload maxima and terrestrial storms is the assumed correspondence of time-displaced events near the end of April. Preliminary comparison of unsmoothed data with terrestrial observatory magnetograms indicates that the extreme phase difference discernible in Fig. 2 for 'corresponding' events between April 21

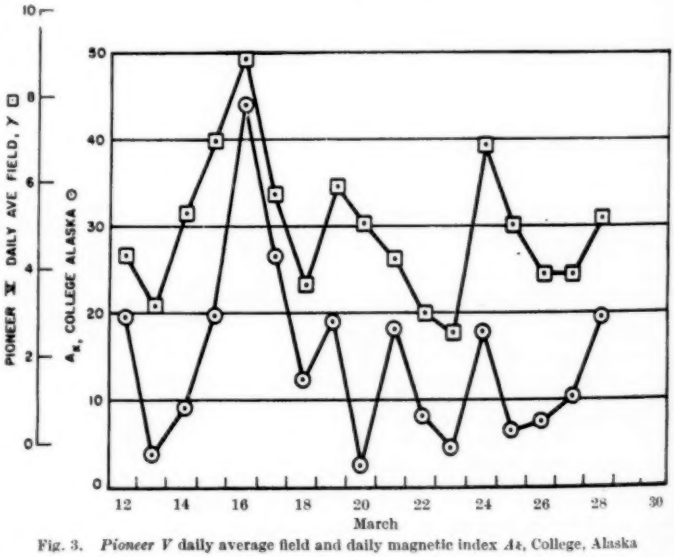

Fig. 3. *Pioneer V* daily average field and daily magnetic index A_k, College, Alaska

and 29 cannot be accounted for entirely by the smoothing process, but is a real feature of the observed interplanetary and geomagnetic fields. Examination of unsmoothed data has also revealed that terrestrial effects were either delayed or persisted longer than corresponding interplanetary disturbances for some events earlier in the history of the flight, although never with phase differences as large as that seen late in April.

An increasing delay toward the end of the period of acquisition of data might be expected to result from the steadily advancing distance between Earth and probe, on the assumption the satellite was intercepting disturbances of solar origin travelling toward the Earth. However, based on the approximate 1,000 km./sec. average velocity usually inferred for transient solar-terrestrial magnetic effects, the expected delay between *Pioneer V* and the Earth, even for distances in effect at the end of April, would be less than 3·3 hr., and thus imperceptible in the scale of Fig. 2. Long delays due solely to increase in time of probe-Earth passage would require local velocities close to 100 km./sec. to account for the observed shifts, therefore ruling out solar effects moving by the payload at ten times this speed.

Suggested interpretations of the time displacements include a low payload-Earth velocity and a terrestrial delay mechanism, and the possibility that excursions observed in the interplanetary region and at the surface of the Earth are related respectively to different phases of the travelling storm phenomenon. Further investigation of the data is now in progress.

Acknowledgment is due to Dr. E. J. Smith and Dr. J. M. Kelso for useful discussions and to M. Saxe for help in preparation of the data used above. P. J. Coleman, jun., was responsible for reduction of the *Pioneer V* magnetic data.

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OBITUARIES

Prof. W. T. Astbury, F.R.S.

WITH the death of Prof. W. T. Astbury on June 4, at the age of sixty-three, the world of crystallography, fibres and molecular biology lost an international figure.

A product of the 'Five Towns', eldest son of a large family, versatility at Longton High School earned a scholarship to Jesus College, Cambridge, where, with a break by service in the Royal Army Medical Corps, he graduated in physics in 1921.

The first phase, 1921–28, of his working life was one of tutelage and growth as assistant to Sir William Bragg, first at University College, London, then, from 1923, at the Davy Faraday Laboratory of the Royal Institution where "in that dawn 'twas bliss to be alive", the happy coterie including Müller, Shearer, Bernal, Yardley, Robertson, Robinson and Cox. There he learnt the lessons of simple and direct experiment, the fine tradition of 'Friday Evening' exposition, the value of collaboration. Notable early work on essential systematics and technique of X-ray crystal analysis were the famous space-group tables with Yardley and the α-ray integrating photometer. Bold attempts at key structures like tartaric acid and the acetyl acetones were necessarily sketchy; introduction to his life-work came in preparing fibre X-radiograms for Bragg's lectures "On Common Things".

In 1928, Astbury found a field ready for his talents as lecturer in textile physics, University of Leeds, Bragg sustaining him in early difficulties. Five years solid work produced papers in the *Philosophical Transactions of the Royal Society* on the structure of hair, wool and related fibres which laid for a generation the foundations of high-polymer structural studies. Briefly, inspired analysis of the vague X-ray diffraction pictures of biological fibres under varied conditions of humidity, temperature, tension and chemical treatments, related to optical, mechanical, chemical and technical features, led to a broad scheme of keratin structure: the $\alpha \rightleftharpoons \beta$ polymorphic transition as reversible intra-molecular chain folding, the folds plausibly related to side-chains and S—S links to explain 'set' and supercontraction. This brought academic and industrial recognition, welcome financial support, and award of the Royal Institution's quaintly worded Actonian Prize.

Extension to muscle and collagen established two broad classes of fibrous protein structure, while similar work on egg albumen and on protein layers emphasized chain folding as a key to fibrous and globular protein relations. These, with *ad hoc* studies of dyeing and tanning and his 'missionary' *Fundamentals of Fibre Structure*, impressed old trades with new knowledge. It was the creative period.

When not abroad, the rest of his life, from 1955 in doubtful health, was spent in Leeds developing such themes, as reader in 1937 (later honorary) and, from 1945, as professor of biomolecular structure. Polypeptides, cellulose, alginates; the vital structures of flagella, nucleic acids, muscle components; excursions on the abnormalities of cancer, influenza, rheumatism or '*pili torti*'; new tools; all was his oyster. About 150 publications in some thirty journals indicate his range and energy. His star shone brightly afar, attracting in all benefactions exceeding £100,000. Rather silent among his peers, he yet gave generous service to governmental and industrial planning committees, to the councils of scientific and professional bodies, to editorial boards, international conferences, industrial consultancy; and to furthering the work of others. Honours coming thick included an honorary D.Sc. Strasbourg, distinguished medals and monetary awards, foreign membership of the National Academies in Sweden and the United States, and (with A. V. Hill) honorary founder-membership of the British Biophysical Society. His quick wit and breezy style made him a

popular choice for memorial lectures such as the Procter, Mather, and Sylvanus Thompson.

Astbury's importance lies in the questions he asked and when—stimulus rather than detailed answer. He was an individualist, poet, shy and unsure rather than leader, artistic amateur not scientific professional, Elia his fellow, his strength and weakness the courage and zest the egoisms of a boy, and 'ware the colleague who had to "be of no school and have no master". He brought his findings to market in the green ear, but would not clear the weeds nor suffer the system and technique necessary for the harvest. Inevitably the protein-structural breakthrough was lost, and it hurt. Yet at bottom he had the humility of giants, kindly to failure and to youth, and one warmed to the Croonian and Harvey lecturer who obediently yielded the pavement to a youthful fellow-dreamer chugging along his imaginary track. If he relished the quip 'physicists and stamp-collectors' his view of biological research was latterly more akin to Hinshelwood's than to Rutherford's.

"I have ventured,
Like little wanton boys that swim on bladders,
This many summers in a sea of glory,
But far beyond my depth."

He would rest content with the simple tribute 'salute to adventurers'. Married in 1922, he leaves a widow, son and daughter. I. MacArthur

When I joined Sir William Bragg's team in the Department of Physics at University College, London, in 1922, W. T. (Bill) Astbury was decidedly the most colourful personality there. He took me under his wing and helped me in every possible way. I think it was partly his natural kindness and partly the fact that his intense happiness in his recent marriage to a lovely young Irish girl made him feel fatherly towards all young people. He was so full of enthusiasm for his work that none of us could feel it to be drudgery, even though it entailed sitting for hours on end with one eye glued to a microscope taking readings of the movements of a gold leaf. He was so delightfully pleased with his own achievements, not only then but throughout his life, that it was impossible not to rejoice with him; and he did achieve miracles with what would have seemed to most people almost intractable problems. Any discussion in which he took part, whether it was about crystal symmetry or about politics, became at once a matter of the greatest interest; any game that he played (and especially our lunch-hour international table tennis tournaments at the Royal Institution) became the more lively because he took part: the normal game was often too slow for him, and he would initiate some twist (five marks extra for hitting the matchbox off the table) to make it more fun. I count myself fortunate to have been one of his friends and colleagues.

Kathleen Lonsdale

Mr. W. V. Lewis

William Vaughan Lewis, lecturer in geography at Cambridge and Fellow of Trinity College, was killed in a motor accident at De Witt, Iowa, on June 8. His work gave him a high standing among geographers in Britain and abroad. With his exceptional eloquence and vitality, he was a born teacher. He was a good friend and had an enduring influence on the lives of many people. His early death comes as a great shock.

Vaughan Lewis was born at Pontypridd in 1907, and was educated at Pontypridd County School. He went up to Gonville and Caius in 1926 and so began a life-long connexion with Cambridge. He read Part I of the Mathematical Tripos, then turned to geography, finding his enduring interest in physiography. After completing the Tripos in 1929 he remained, first as a student, and then as a University demonstrator. He became a lecturer in 1945, when 'freezing' of University appointments ended. In 1949, to his great pleasure, he was elected a Fellow of Trinity College.

Vaughan Lewis's research career began when Prof. (then Mr.) Steers took him to Scolt Head Island. He was intrigued immediately with problems of coastal deposition and morphology, turning his own attention to Dungeness. He was engaged mainly with coastal research, until in 1936 he visited Iceland and became a confirmed glaciologist. While this was the field of his greatest influence, he typically enlarged, rather than changed, his interests, later doing much research on Chesil Beach, the results of which remain to be published. He had also a secondary interest in hydrology.

His glaciological work, carried out mainly in Norway and Switzerland, was concerned particularly with aspects of the structure, flow and erosive influence of cirque and valley glaciers. Apart from his published contributions to glaciology, such as his lively exchanges on the cirque problem with D. W. Johnson, it should be recorded that he was personally responsible for making physicists interested in field-work on glaciology. He played a leading part in the affairs of the British Glaciological Society, which has much enhanced latterly the active development of glaciology in Britain and elsewhere. Vaughan Lewis initiated two intensive research projects in Norway. The results of the earlier investigation of cirque glaciers were published in a research memoir of the Royal Geographical Society, of which he was the editor. His personal interests in the Austerdalsbreen Valley glacier project are recorded in a recent joint paper in the *Glaciological Journal*. These investigations depended on the co-operation of numbers of workers in different fields, and on the active support of generations of Cambridge undergraduates and research students. Vaughan Lewis again and again caught the real interest of undergraduates and, naturally, gathered around him eager groups of volunteers for field-work. The range and vividness of his ideas were such that he had a catalytic effect on the work of others. He was always generous, and would continue to encourage and assist those whom he had inspired to undertake some investigation. He will be sadly missed in many places. Jean M. Grove

Mr. Geoffrey Parr

With the death of Geoffrey Parr on May 30 at the comparatively early age of sixty-one, a colourful and popular figure leaves the scientific world. Parr received his formal education as an electrical engineer at the Finsbury Technical College, and, after working for the Admiralty during the latter part of the First World War, became a lecturer and demonstrator at the City and Guilds Technical College. In 1926 he joined the Research Department of the Edison Swan Electric Co., Ltd., where the cathode-ray tube was undergoing some of its early developments. Then in 1932 he took charge of the same Company's Technical Service Department.

His real *métier* and flair, however, appeared to lie in the communication of knowledge—writing, publicizing, lecturing, editing and publishing.

In the middle 'thirties he published his first book, *The Cathode-Ray Tube and Its Applications*, which has since gone into many editions and become a standard work.

In 1941, Parr became the first editor of *Electronic Engineering* and did much to nurture that paper through its early phases and war-time difficulties. In 1948 he joined Messrs. Chapman and Hall, Ltd., as technical director, and remained in that appointment until his death, being responsible for the very considerable output of scientific books from this well-known house.

Recently, Parr wrote and published, in collaboration with Mr. J. Godfrey, *The Technical Writer*, a work which filled a much-felt void and should long remain an authentic guide to writing standards among scientific authors.

Apart from his professional work, Geoffrey Parr's 'side' activities were legion and these helped to absorb his boundless energy and enthusiasm. One of these was his association with Dr. Grey Walter in the development of the electro-encephalograph, for which Parr helped in the design and construction of the electronics and amplifiers, the whole forming the basis of a joint paper to the Institution of Electrical Engineers, entitled "Amplifying and Recording Techniques in Electro-biology" (*J. Inst. Elec. Eng.*, **90**, 3; 1944).

He also lectured frequently to technical colleges and societies throughout the country, usually on some aspect of television. Parr interested himself in many scientific societies and helped in the practical management of several of them. Among these, and in particular, was the Television Society of which Parr had been a Fellow for nearly thirty years, and to which he devoted so much paternal care and devotion. Becoming honorary lecture secretary in 1936, honorary editor of the *Journal* in 1944 and honorary secretary in 1945, he gave unstintingly his free time and attention before, during and since the War years. In fact, he had only retired from the office of honorary secretary a fortnight before his death. He helped the Society survive the War and then grow into its present stature, and, to a large extent, he personally fostered all the important aspects of its development.

Geoffrey had a host of friends in the world of television, in technical publishing and journalism, and wherever men of science gathered; for his gaiety, informality and humanity hundreds will remember him gratefully and affectionately.

T. H. BRIDGEWATER

NEWS and VIEWS

Pure Mathematics at Aberystwyth : Prof. V. C. Morton

PROF. V. C. MORTON retires in September from the chair of pure mathematics at Aberystwyth after thirty-eight years on the staff of the University College of Wales. Educated at King Edward VII School, Sheffield, and Merton College, Oxford, of which he was a scholar, he served in the Royal Corps of Signals during 1916–19. On his return he became Junior and Senior Mathematical Scholar of the University of Oxford. He lectured for a year at Brighton Technical College and in 1923 was appointed lecturer at Aberystwyth. He became independent lecturer and head of the Pure Mathematics Department in 1926 and professor in 1933. He investigated various geometrical configurations, his best-known work being contained in a series of papers on the cubic surface. Dean of the Faculty of Arts, twice dean of that of Science and vice-principal, he has served the College and the University of Wales in many capacities, most notably as acting-principal of the College in 1957–58. A gifted teacher and a stimulating lecturer, wise in counsel and disinterested in judgment, his integrity and magnanimity are an inspiration to his students and colleagues alike.

Prof. W. B. Pennington

DR. W. B. PENNINGTON, who has been appointed to succeed Prof. Morton, is thirty-eight years of age. He had a distinguished career in Cambridge culminating, after interruptions by war service in the R.N.V.R. (1943–46) and two years at Harvard University (1950–52), in a Research Fellowship at Jesus. Since 1953 he has been reader in mathematics at Westfield College, University of London. Dr. Pennington is well known to British mathematicians, not only for his contributions to the analytical theory of numbers, but also for his devoted activities in the service of the London Mathematical Society as member of Council, vice-president and *Journal* editor. With his forward-looking attitude to mathematics and the catholic range of his interests, extending to problems of mathematical teaching and administration no less than of research, Dr. Pennington has not stood aloof from the heart-searchings prevalent among mathematicians to-day, and his new appointment will enhance his opportunities for an effective part in the great debate. With his departure to Wales, Westfield College loses an able mathematician, a delightful colleague, an opening batsman and an accomplished baritone.

Physical Chemistry in Trinity College, Dublin: Prof. D. C. Pepper

DR. DAVID CHARLES PEPPER has been appointed to the newly established chair of physical chemistry in Trinity College, Dublin. Prof. Pepper, born in 1917, was educated at Ushaw College, Durham, Bradford Technical College, from which he took the London degree of B.Sc., and Jesus College, Cambridge. Later, he was awarded the Cambridge degree of Ph.D. for work in the Department of Colloid Science in Cambridge. He was appointed assistant lecturer in Trinity College, Dublin, in 1945, and Fellow in 1951. He has been reader in physical chemistry since 1955, and has been a member of the Royal Irish Academy since 1953. Prof. Pepper's research career commenced with Sir Eric Rideal, with whom he worked on a variety of war-time topics, sponsored by the Scientific Advisory Council of the Ministry of Supply. These researches were concerned

XUM

—among others—with interactions between phenols and amine monolayers, catalytic oxidation of ethylene, the plastic and vinoelastic behaviour of solid–liquid dispersions and polymer gels, and the kinetics and mechanism of polymerization reactions. The last-named topic has formed the basis of Prof. Pepper's interests in Trinity College, Dublin, most of his work being in the field of polymer solutions or in studies of the kinetics and mechanism of cationic polymerization. His research school has revealed some of the special characteristics of these reactions, notably the effect of solvents both through their dielectric behaviour and as co-catalysts. His work has focused attention on the considerable kinetic differences between these ionic polymerizations and free-radical polymerizations. It is true to say that contemporary theory in this field is largely based on ideas developed in Prof. Pepper's research group.

U.S. National Bureau of Standards: New Appointments:

Mr. Martin T. Decker

Mr. Martin T. Decker has been appointed chief of the Tropospheric Measurements Section of the National Bureau of Standards Laboratories at Boulder (Colorado). Mr. Decker joined the Bureau staff in 1949, his early work being in the area of tropospheric forward scatter of radio waves. More recently his research has been primarily in air-to-ground communications, including air navigation and airborne television studies, especially the prediction of reliability. He has also made studies of the obstructing effects of various materials on radio waves at VHF, UHF and SHF. Born in Byron, Nebraska, in 1927, Mr. Decker received his B.S. degree in electrical engineering from the University of Nebraska in 1950. He has continued his engineering studies at the University of Colorado.

Mr. John L. Hague

Mr. John L. Hague, a national authority in the field of analytical chemistry, has been appointed chief of the Standard Reference Materials Section. Mr. Hague had been acting chief of the predecessor section, Analytical Chemistry, since March 1960. The Standard Reference Materials Section will play an important part in the Bureau's standard materials programme. In this programme, the Bureau makes available for distribution to science and industry more than 550 different standards of metals, ores, ceramics, chemicals, hydrocarbons, and more recently added radioactive materials, uranium isotopes, lithium ores, aluminium refractories, and phosphor materials. Standard materials are used to check, evaluate and standardize methods of analysis and analytical techniques and substances, and to develop new and improved methods and instruments. Mr. Hague joined the Bureau staff in 1930 as a laboratory apprentice. In 1932 he transferred to the Norfolk Shipyard and three years later was in charge of the Foundry Control Laboratory for ferrous and non-ferrous alloys. He returned to the National Bureau of Standards in 1935 and in 1951 became assistant chief of the Analytical Chemistry Section. Born in Kansas City, Missouri, in 1912, Mr. Hague received his B.S. degree in chemistry in 1938 from George Washington University and has done graduate work there. In 1958, Mr. Hague received the Department of Commerce Meritorious Service Award "for outstanding original contributions to methods of analysis necessary for controlling the production of complex metal alloys".

Mr. John K. Taylor

Dr. John K. Taylor, research chemist, whose entire 32-year professional career has been with the National Bureau of Standards, has been appointed chief of the Applied Analytical Research Section of the Bureau's Analytical and Inorganic Chemistry Division. The Applied Analytical Research Section is concerned with the development and application of methods for the analysis and characterization of solids, liquids and gases. Both classical and instrumental methods of analysis are employed, using both macro- and micro-techniques. The Section also provides analytical consultation services and assistance for other Bureau programmes. A major part of Dr. Taylor's work at the National Bureau of Standards has been concerned with the application of physical methods to chemical analysis, including the polarographic method and the limitations of voltammetric processes. Recently he has conducted research in high-precision coulometric methods of analysis. Dr. Taylor has written numerous papers on these subjects, and in 1960 was awarded the Department of Commerce Silver Medal in "recognition of his contributions to accurate electrochemical methods of analysis". Other areas of research interest for Dr. Taylor have included refractometry methods for separation of isotopes, and a programme for providing a series of uranium isotope standards. This last has consisted of high-precision analysis of uranium compounds and the blending of highly purified isotopic species for mass spectrographic analysis. Born in Mt. Ranier, Maryland, in 1912, Dr. Taylor received his B.S. in chemistry from George Washington University in 1934. He was awarded his M.S. and Ph.D. from the University of Maryland in 1938 and 1941, respectively. Throughout his professional life, Dr. Taylor has been an active supporter of science education. He is a member and past chairman of the Joint Board on Science Education of the Washington Academy of Sciences and is editor of *The Reporter*, a news-letter for science and mathematics teachers.

Public Investment and Export

Under the title "Public Investment and Export" an article by Sir Walter Benton Jones, Bart., which originally appeared in the *Times Review of Industry*, March 1961, has been, with the addition of some figures for public spending 1950–59, issued as a pamphlet (Pp. 7. Grantham: Sir Walter Benton Jones, Irnham Hall, 1961). Sir Walter asks the leading question: Has Britain reached a level of public spending which may jeopardize her power to compete in the expanding export markets of the world, which may soon become more competitive than at any time in history? Pointing out that public spending has risen from £3,692 million in 1950 to £5,099 million in 1955 and £6,237 million in 1959, whereas exports increased by 33 per cent in the first period and only 16 per cent in the second, compared with increases of 49 per cent and 20 per cent, respectively, in world exports, 52 per cent and 13 per cent, respectively, for the United States, and 21 per cent and 33 per cent for West Germany, he suggests that the high level of our public spending may be handicapping our exports. Sir Walter suggests that since Parliament ultimately fixes and controls public expenditure, Parliament should be concerned to regulate the level of public expenditure by public income after due regard to possible effects of taxation on production and export, and that what is primarily

needed to stimulate export is a considerable easing of the burden of taxation. He does not suggest any particular reductions in expenditure and admits that much expenditure is desirable or essential, but the argument is one with implications which the scientist might be wise to consider.

Nutrition Society of Canada

At the fourth annual meeting of the Nutrition Society of Canada, held at the Ontario Agricultural College, Guelph, on May 30, the following officers were elected: *President*, Dr. E. H. Bensley, Montreal; *Vice-President*, Dr. R. H. Common, Macdonald College; *Treasurer*, Dr. J. A. Campbell, Ottawa; *Secretary*, Prof. E. V. Evans, Guelph; *Councillors*, Dr. J. M. Bell (1964), Saskatoon, Dr. L. P. Dugal (1963), Ottawa, and Dr. W. W. Hawkins (1962), Halifax. A feature of the annual dinner of the Society was the announcement of the winner of the second Borden Award of the Nutrition Society of Canada for research in nutrition. This year's winner, Dr. Donald Fraser, of the Research Institute, Hospital for Sick Children, Toronto, Ontario, was cited for his work on vitamin D-refractory rickets in children and on varied aspects of calcium and phosphorus metabolism. The special speaker at the dinner was Dr. Ross A. Chapman, assistant director, Scientific Services, Food and Drug Directorate, Ottawa, whose topic was "Research in the Food and Drug Directorate". The one-day Society programme included a session of short research papers and a symposium on "Current Problems in Nutrition".

New Thinking in School Mathematics

A revision of the school curriculum in mathematics is needed for two main reasons. First, the gap between school mathematics and new mathematics is increasing, though it is more than ever important that the average intelligent citizen should understand that mathematics is the true foundation of our technological society. Secondly, the shortage of mathematics teachers might be partially remedied by ensuring that no time is wasted by teaching valueless or outmoded material. The Organization for European Economic Co-operation arranged a survey and a seminar at Royaumont attended by about fifty representatives from Europe and North America. A report, edited by Howard Fehr, is now available (Pp. 246. Paris: Organization for European Economic Co-operation, 1961. 10 N.F.; 15*s*.; 2.50 dollars; 8.50 D.M.). Part 1 summarizes the proceedings of the seminar. There was a general demand for a reduction in old-fashioned geometry, the inclusion of modern ideas in algebra, possibly with an abstract basis, and the introduction of probability and statistics. The implementation of such a programme would require new text-books and equipment, revised courses for teacher-training, and the re-education of serving teachers. Part 2 gives the results of the survey of present practices and trends, with much valuable information concisely presented. The volume should be studied carefully by all teachers of mathematics; even if they quail at some of the ultra-radical suggestions, they will appreciate the insistence on pattern and structure as the essence of mathematics.

Coryndon Memorial Museum, Nairobi

The annual report of the Coryndon Memorial Museum for 1959 is, as usual, a record of much progressive work. This included the establishment of a Snake Park and Snake Study Centre surrounded by a Botanical Garden, to which there was immediate popular approval (Pp. 24+1 plate. Nairobi: Museums Trustees of Kenya and Coryndon Memorial Museum, 1961. 1*s*.). All Departments of the Museum are in urgent need of cabinets for storage, especially in view of the almost indescribable dust problem in dry, windy weather. A number of new exhibits was completed. These include a habitat group depicting the swordfish, two giant fossil human milk teeth from the Olduvai Gorge and a leopard habitat group.

Western Australian Museum

The annual report of the Western Australian Museum for the year ended June 30, 1960, records the separation as from May 27, 1960, of the Museum and the Art Gallery. The Trustees of the combined institution have been replaced by two Boards of five members each. Consequent on this separation the Art Gallery moved out of the present James Street wing. Appropriately, the report includes an illustrated short historical sketch of the Museum, together with a comprehensive statement regarding future policy in relation to the various Departments. An account is also given of the major expeditions undertaken during the year, and these include one to investigate the fauna and flora of the Bernier and Dorre Islands in Shark Bay, another to Northwest Cape, and assistance given to the Bernice P. Bishop Museum of Hawaii when it had a mollusc-hunting expedition in Western Australia. The practice of including a short summary of papers published by members of the staff is useful and worthy of commendation.

Treatment of Varicose Veins

Varicose veins of the lower extremities are a frequent and highly disabling condition. Their surgical management has undergone a great number of variations since varicosities were treated by puncturing and drainage in the Hippocratic era. The exact ætiology of varicose veins still remains obscure; the disease may tend to be progressive, and morbidity causes economic strain on the patient and the community. Yutaka Kato and Robert A. Nabatoff, of the Department of Surgery, the Mount Sinai Hospital, New York City, N.Y., have carried out complete stripping of varicose veins of the lower extremities under local anæsthesia (*Yokohama Med. Bull.*, 11, No. 6; December 1960). This permits, almost immediately, post-operative ambulation and minimizes thrombo-embolic phenomena. The number of incisions depends on the number of incompetent perforator veins. All incompetent perforators are accurately mapped out pre-operatively, and all are ligated flush with the deep veins prior to the stripping procedure. Since all the veins communicating with the diseased great saphenous system and all 'blow-outs' in other locations are interrupted, and since the remaining varicose vein segments are stripped out, very few, if any, injections of sclerosing solution are necessary following operation.

The American Philosophical Society

Year Book 1960 of the American Philosophical Society contains, besides the lists of present and former officers of the Society and of former and present members, the charter and laws of the Society, and a brief history of the Society by E. G. Conklin (Pp. 786. Philadelphia: American Philosophical Society,

XUM

1961). The largest of the reports of committees is that of the Committee on Research, which includes lists of the 170 grants totalling 165,609 dollars made in 1960 from the Penrose Fund and of the 53 grants, totalling 53,473 dollars, made from the Johnson Fund. Four fellowships, totalling 27,400 dollars, were awarded from the Darland Fund. Reports from recipients of grants occupy almost 400 pages of the 416 of the Committee's report. Among the biographical memoirs included in the volume may be mentioned those on R. C. Andrews, W. H. W. Baade, N. L. Bowen, O. E. Buckley, A. L. Day, Sir Walter Wilson Grey, M. von Laue, L. Loeb, S. A. Mitchell and E. C. Tolman.

Cigarette Smoke Condensate

The Tobacco Manufacturers' Standing Committee has published a second edition of its Research Paper No. 4, *Cigarette Smoke Condensate: Preparation and Routine Laboratory Estimation* (by H. R. Bentley and J. G. Burgan. London, 1961). This largely follows the first edition published in 1959, which it supersedes; but in the new edition the section referring to routine laboratory estimation of the yield of cigarette smoke condensate has been revised and extended. The standard method given in the first edition has been modified in the light of experience in order to improve the reproducibility of results. In particular, collection of smoke particulate matter by means of a 'Cambridge' filter followed by drying to constant weight is recommended for general use on grounds of simplicity, in preference to electrostatic precipitation, which has been found to have some disadvantages for quantitative work. Consideration is also given to the definition of realistic smoking conditions to be used with automatic smoking machines, and parameters, based on observations of smokers, of one puff of 2-sec. duration and 25-ml. vol./min., are recommended. These values are useful to have, for it would clearly be an advantage if some such agreed parameters were to be adopted in all laboratories in the United Kingdom in which cigarette smoke is being prepared and investigated.

Colour in Factories

Colour in industry is not merely a form of decoration or even a welfare measure. It has certain functions to perform and the factors important for success have been established. The latest report in the Factory Building Studies series, based on several years work on the use of colour in various types of building, is a concise guide for factory managements and their architects or colour consultants (Factory Buildings Studies No. 8. *Colouring in Factories*. Published for D.S.I.R. by H.M.S.O. Price 7*s*. 6*d*., postage 4*d*.). It has two sections, both containing coloured illustrations. The first section describes the basic properties and effects of colour, linking them with certain facts about human eyesight, to form general principles of colour design. The second section explains the practical application of these principles. It indicates stage by stage the way in which colour for each element of the interior becomes disciplined so that a picture is formed of the characteristics essential to good factory colouring. The examples are mainly for daylight, but there are notes on the special problems arising where lighting is wholly or partly artificial. As lighting and colouring are interrelated, this work is complementary to the earlier work (No. 2 in the series) on *The Lighting of Factories*.

The Content of Jobs

To the steadily growing number of broadsheets which are being produced to assist all who are concerned with the personnel function of management, the Institute of Personnel Management has now issued one called *Job Analysis*. Prepared by H. E. Roff and T. E. Watson, of Management Selection, Ltd., the broadsheet defines job analysis as "the scientific study of statements of all the facts about a job which reveal its content and all the modifying factors which surround it". Accepting that much of what passes for job analysis to-day is scarcely scientific, the authors show how a systematic approach to the analysis of a job can help all those who have to deal with human problems arising from the establishment of wage or salary scales, and the intricacies of selection, training and safety. Great difficulties confront industrial management in classifying one job against another and the method described in *Job Analysis* provides a useful *modus operandi*. For detailed information the reader must seek elsewhere, and the broadsheet contains a valuable bibliography which will help him to do this. *Job Analysis* is obtainable from the Institute of Personnel Management, Management House, 80 Fetter Lane, London, E.C.4, price 7*s*. 6*d*.

European Organic Geochemistry Group

At a recent meeting of members of various European geochemistry groups it was proposed that a European section of the Organic Geochemistry Group of the Geochemical Society should be formed. At present the Organic Geochemistry Group has 250 members, 45 of whom are resident in Europe. The Group held very successful meetings in Denver last autumn, and this year meetings are to be held in Cincinnati. As such, countries interested in such an organization include Italy, France, the United Kingdom, Germany and Holland. The possibility of a meeting in Milan during September 1962 is being considered. Persons interested in such an organization should write to Dr. G. D. Hobson, Department of Geology, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7.

Oliver Memorial Fund Award for Blood Transfusion Work

The Committee of the Oliver Memorial Fund intends making its fourteenth annual award of the value of £50 to a British subject whose original work, or services in connexion with blood transfusion, is considered to be a notable contribution to the research organization or donor aspect of this subject. The Committee will welcome applications and, in addition, communications directing its attention to suitable candidates. Applications should be submitted before September 30, and should be sent to the honorary secretary, Mrs. F. E. Bedborough, 105 New Park Avenue, London, N.13.

Australian Post-Doctoral Fellowships in Protein Chemistry

The Division of Protein Chemistry of the Commonwealth Scientific and Industrial Research Organization, Australia, is offering a number of post-doctoral fellowships. The Division has been engaged on a comprehensive research programme on protein structure and chemistry for the past twelve years covering chemical, physicochemical, biophysical and

histological studies. The Division is well equipped for work in these fields and the applicant will be able to work with either fibrous or globular proteins. The fellowships have a three-year tenure and provide fares for the Fellows and their families to and from Australia. Salaries of up to £A2,720, depending on experience, are offered. Further information can be obtained from C. Garrow, Division of Protein Chemistry, C.S.I.R.O., Wool Research Laboratories, 343 Royal Parade, Parkville, N.2, Victoria.

Ramsay Memorial Fellowships

THE Ramsay Memorial Fellowships Trustees have made the following awards of new fellowships in chemistry for the year 1961–62: a general fellowship to Mr. M. J. How at the University of Birmingham; a Japanese fellowship to Dr. H. Suginome at the University of Cambridge; a New Zealand fellowship to Mr. B. A. Grigor at the University of Leicester; a Spanish fellowship to Mr. Fernan Alonso Matthias at the University of Cambridge; United States fellowships to Dr. K. D. Carlson at the University of Oxford and Dr. D. W. Setser at the University of Cambridge. The Trustees have renewed the following fellowships for the same year to: Mr. A. Bewick (general fellowship) at the University of Durham; Dr. D. G. Watson (Glasgow fellowship) at the University of Cambridge; Mr. H. Krabbendam (Netherlands fellowship) at the University of Edinburgh; Mr. G. A. Rodley (New Zealand fellowship) at University College, London; and Dr. Maria Covadonga Rodriguez (Spanish fellowship) at the University of Oxford.

Statistical Quality Control and Acceptance Sampling

THE College of Advanced Technology, Birmingham, is organizing a full-time course of three weeks duration on "Statistical Quality Control and Acceptance Sampling", commencing September 11. The course is primarily intended as an introduction to the subject. Nevertheless, it provides the basic training necessary for quality control engineers. The first fortnight will be spent in covering the general principles and procedures of quality control. The final week will be devoted to lectures by specialists from industry, and to visiting the works of firms using quality control, where members of the course will be able to see the practical application of quality control methods, and discuss the techniques and associated problems. Further information can be obtained from the Bursar, College of Advanced Technology, Gosta Green, Birmingham 4.

Food Science Conference

THE new Food Research Laboratories for the Commonwealth Scientific and Industrial Research Organization, Australia, are being opened at North Ryde, near Sydney, on September 18, and, to mark the occasion, a conference on "Food Science" will be held there during September 19–22. The conference will take the form of three symposia: "Chemical Aspects of Food Processing"; "Factors affecting Meat Quality"; "Food Preservation and the Organization of Plant and Animal Tissues". Further information can be obtained from the Chief, Division of Food Preservation, C.S.I.R.O., Private Bag, Homebush Post Office, N.S.W., Australia.

University News : Birmingham

THE following appointments have been announced: Dr. L. Riddiford, senior staff Fellow in nuclear physics, to be senior lecturer in the Department of Physics; G. Martelli, staff Fellow in nuclear physics, to be lecturer in the Department of Physics; Dr. S. Swierszczewski, to be research Fellow in nuclear physics in the Department of Physics; Dr. J. H. Broadhurst, to be senior research associate, and D. E. Kidd, to be research associate, in nuclear physics in the Department of Physics; Dr. C. A. Barson and Dr. A. E. Pedler, to be senior research Fellows in the Department of Chemistry; R. J. Heitzmann, to be research Fellow in the Department of Chemistry; R. G. Bird, to be lecturer in the Department of Civil Engineering; B. K. Gazey, to be research Fellow in the Department of Electrical Engineering; Dr. B. Henderson, to be research Fellow in the Department of Physical Metallurgy; G. R. Ogram and B. Walker, to be research Fellows in the Department of Industrial Metallurgy; Dr. A. T. Kolnai, to be Lloyd-Muirhead Fellow in social philosophy in the Department of Philosophy, for one year; J. R. Turcan, staff tutor in the Institute of Engineering Production, to be research Fellow in the Department of Engineering Production; Dr. B. J. Key, to be lecturer in neuropharmacology in the Department of Experimental Psychiatry; Dr. R. R. Dils, to be lecturer in the Department of Medical Biochemistry and Pharmacology; D. Rimmer, to be lecturer in the Department of Economics.

Edinburgh

J. H. TURNER has been appointed as lecturer in pathology. The following have been appointed Imperial Chemical Industries Research Fellows for a period of three years, with effect from October 1: Dr. I. Bihler (Hebrew University); Dr. J. Newham (Hull); C. McGreavy (Yale); Herman Wen-Geun Chew (Chicago); Dr. A. S. Milton (Oxford).

London

DR. M. GORDON, senior lecturer in physical chemistry at the Imperial College of Science and Technology, has been appointed to the University readership in physical chemistry tenable at that College. The title of reader in demography in the University of London has been conferred on Mr. J. Hajnal, in respect of his post at the London School of Economics and Political Science.

Manchester

ON an initiative jointly taken by Unilever Ltd. and the Department of Economics of the University, a Centre for Business Research is to be set up in Manchester. The Centre, which will be financed by money from industry, will, with the assistance of the co-operating firms, study some of the difficult problems of business decisions, and the ways in which economic, statistical and econometric knowledge can be used to help the decision-making of British business. Support has been guaranteed for an initial period of seven years, and it is hoped to appoint the first members of the full-time staff within a few months. The project will be directed by Prof. C. F. Carter, J. Johnston and B. R. Williams.

Sheffield

DR. N. H. MARCH, at present reader in theoretical physics, has been appointed to the newly created second chair of physics as from October 1.

The following new appointments have also been made: Dr. V. C. L. Hutson, to be lecturer in applied mathematics; J. W. Ross, to be junior research

Fellow in physics; Dr. I. Hawkes, to be lecturer in the Postgraduate School of Mining; Dr. C. J. Ball, to be lecturer in metallurgy; I. Livingstone, to be lecturer in economic statistics; P. E. Brown, to be senior lecturer in preventive medicine and public health; W. T. Baker, to be health physicist.

The Night Sky in August

New moon occurs on Aug. 11d. 10h. 36m. U.T. and full moon on Aug. 26d. 03h. 14m. The following conjunctions with the Moon take place: Aug. 5d. 17h., Aldebaran 0·3° S.; Aug. 7d. 18h., Venus 3° N.; Aug. 14d. 18h., Mars 2° S.; Aug. 23d. 15h., Saturn 3° S.; Aug. 23d. 23h., Jupiter 3° S. In addition to these conjunctions with the Moon, Venus is in conjunction with Pollux on Aug. 24d. 11h., Venus being 7° S. There will be an annular eclipse of the Sun on Aug. 11, invisible at Greenwich. There will be a partial eclipse of the Moon on Aug. 26, visible at Greenwich. The Moon enters penumbra at 0h. 36·1m., enters umbra at 1h. 34·9m., leaves umbra at 4h. 41·5m., and penumbra at 5h. 40·4m., the magnitude of the eclipse being 0·992. Moonset at Greenwich on Aug. 26 is at 5h. 11m. Mercury is too close to the Sun for observation. Venus is a morning star, rising at 1h. 05m., 1h. 15m., and 1h. 50m. on Aug. 1, 15 and 31, respectively. Its stellar magnitude on Aug. 15 is − 3·5; the visible portion of the apparent disk increases from 0·683 to 0·792 during the month and its distance from the Earth increases from 96 to 115 million miles. Mars is too close to the Sun for observation. Jupiter sets at 3h. 50m., 2h. 40 m. and 1h. 30m. at the beginning, middle and end of the month, respectively. It is near the border of Sagittarius and Capricornus; its stellar magnitude on Aug. 15 is − 2·3 and its distance from the Earth 385 million miles. Saturn sets at 3h. 20m., 2h. 15m. and 1h. 10m. on Aug. 1, 15 and 31, respectively. It is in Sagittarius; its stellar magnitude on Aug. 15 is + 0·4 and its distance from the Earth is 845 million miles. There are no occultations of stars brighter than magnitude 6 visible at Greenwich. The Perseid meteors are active during the first half of the month. Maximum activity occurs on Aug. 12, the radiant is at R.A. + 3h. 04m., Dec. + 58°, and conditions for observation are very favourable.

Announcements

Dr. Arno Sigvard Eklund, of Sweden, has been appointed director-general of the International Atomic Energy Agency, in succession to Mr. Sterling Cole, whose four-year term of office ends on November 30, 1961. Dr. Eklund was secretary-general of the second United Nations Geneva Conference on the Peaceful Uses of Atomic Energy in 1958, and is chairman of the international board of management of the Organization for European Economic Co-operation High-Temperature Reactor Project *Dragon* at Winfrith Heath, Dorset.

Dr. Morten Simonsen, lecturer in immunology, University of Copenhagen, has been appointed director of research at the McIndoe Memorial Research Unit, Queen Victoria Hospital, East Grinstead, Sussex, which is devoted to the study of tissue transplantation and associated problems of wound healing.

Mr. J. P. Catchpole, of the Department of Chemical Engineering, University of Birmingham, has been awarded the student's prize of the Institution of Chemical Engineers, for his paper entitled "Frequency Response Techniques for the Study of Packed Bed Characteristics". This is the first award of this prize, which is open to student members of the Institution of at least six months standing at the time of presentation or publication of the paper.

Arthur Guinness Son and Co., Ltd., have established a scheme of awards of cash prizes totalling up to £500, to be called the Guinness Awards for Science Teachers in Training. The scheme is administered by *The Science Teacher*, the editorial director of which, Mr. Maurice Goldsmith, is chairman of the Guinness Awards Advisory Committee. The object of the scheme is to encourage students in teacher-training to develop and report on their own original contributions to the pedagogy of science in all its branches. The scheme will begin to operate as from September 1961. Further information can be obtained from the Information Office, Arthur Guinness Son and Co. (Park Royal), Ltd., Berk House, 8, Baker Street, London, W.1.

An international congress of aviation and cosmonautical medicine is being held in Paris during September 26–30. Further information can be obtained from the secretariat, C.E.R.M.A., 5*bis* avenue de la Porte de Sèvres, Paris, France.

The Faraday Society has arranged a general discussion on "The Structure and Properties of Ionic Melts" which will be held in the Department of Inorganic and Physical Chemistry, University of Liverpool, during September 5–7. Further information is available from the Secretary, Faraday Society, 6 Gray's Inn Square, London, W.C.1.

The sixth annual conference of the British Society for the Philosophy of Science is being held at St. Hugh's College, Oxford, during September 22–24. The following are included in the programme: explanation in psychology; learning machines; genetic coding and information theory; statements about the universe. Further information can be obtained from Dr. M. B. Hesse, Whipple Museum, Free School Lane, Cambridge.

The First International Conference on Timber Engineering will be held in the University of Southampton during September 18–23. General problems of timber construction, structural timber joints, structures of particular interest, testing of timber structures, timber shell structures, constructional problems and economics and comparisons are the main subjects which will be discussed. Further information can be obtained from Dr. H. G. Allen, Department of Civil Engineering, University, Southampton.

A series of postgraduate full-time courses is to be held in the Nuclear Studies Section of the Department of Chemistry and Biology, Liverpool College of Technology, during the 1961–62 session. These include a seven-months course in radiation safety and health physics, a number of two-week courses in radiation safety, radiobiology and radiochemistry, and a one-week course in radiation chemistry. Further information can be obtained from the Principal Lecturer and Organizer in charge of Nuclear Studies, Chemistry and Biology Department, College of Technology, Byrom Street, Liverpool 3.

HAZARDS OF THE ANIMAL HOUSE

THE Laboratory Animals Centre continued its series of annual symposia with a discussion on "Hazards of the Animal House" at the Royal College of Surgeons of England, London, on May 30. As a first discussion of this problem, the symposium was concerned with the general assessment of the situation, indicating the risks involved in handling animals suffering from natural and experimental infections and outlining the methods by which these risks might be reduced. Attention was also directed to hazards from sources other than pathogenic organisms, including risks involved in experiments with radioactive material, allergic reactions to experimental animals and traumatic risks due to careless handling of animals.

Since, perhaps, the greatest hazard of the animal house is that the worker should be unaware of the risks to which he is exposed, it is natural that many of the papers dealt with various agents which could be transmitted from animals to man. Early in the symposium, Mr. Bywater referred to the fact that, although the first case of infection of man with *B* virus from a monkey took place in the 1930's, it seemed that the risk had not been appreciated until comparatively recently. Rules for the conduct of animal houses, aimed at the protection of not only the scientific worker but also the animal house staffs themselves, have been drawn up in the past few years. Such rules are of obvious value in dealing also with dysentery and other intestinal conditions, which are transmissible from monkey to man. Dr. Sommerville listed the natural infections of laboratory rats and mice which are transmissible to man. Lymphocytic choriomeningitis has not been encountered frequently in man in Great Britain, although it appears to be fairly common in the United States, sometimes producing an influenza-like syndrome, at other times a biphasic illness with an aseptic meningitis. The encephalo-myocarditis virus of mice is also of reasonably frequent occurrence, but other viruses are rare. On the bacterial side, *Pasteurella septica*, although found both in rodents and in man, has not often been clearly demonstrated as having passed from one to the other. However, since it has caused fatal illness in man, it should be taken seriously. Leptospirosis is also of importance.

A second group of infections is caused by viruses and bacteria transmitted from mice or rats inoculated in the laboratory with diagnostic material or with stock viral or bacterial cultures. In this second group, infection is much more frequent than by viruses and bacteria naturally carried by the animals, but the laboratory worker is usually on the lookout for the agent concerned. The worker may not always be ready, however, to realize the ease with which infection may be transmitted by inhalation or ingestion of dust from cages contaminated with excreta or nasopharyngeal material from the rodents.

Mr. McNeil dealt with the more specialized topic of the hazards associated with farm animals. He emphasized the importance of selection of healthy animals for experimental work both by clinical examination and by application of tests such as tuberculin tests and agglutination tests for *Brucellosis*. A quarantine period on arrival is valuable, since it also allows parasitic conditions such as ringworm, lice or mange to be dealt with. Most of the conditions which affect farm animals are likely to be familiar to the staff engaged in handling these species. Careless post-mortem technique, though perhaps not strictly part of the animal house routine, was referred to as a source of infection in such conditions as anthrax and swine erysipelas. Mr. Hodgman dealt with the hazards encountered in dealing with dogs and cats. He considered that the risks from disease are not of great importance in Great Britain, the main hazard being the traumatic one from bites and scratches. Conditions affecting dogs and cats and transmissible to man are more likely to be apparent in owners of pets than in laboratory workers.

Mr. Garratt spoke of the hazards associated with the use of radioactive substances in animal experiments. His main conclusion was that health hazards to personnel from external irradiations are usually negligible and those resulting from internal irradiation after ingestion or inhalation of radioisotopes could be prevented by careful handling. Careful disposal of waste was of great importance. In general, the attitude of the worker with radioactive substances in animal experiments might be summarized by the maxim: "Take care of your experiment and your health will take care of itself". Other specialized problems were dealt with by Dr. Cooper on the handling of large-scale experiments with polio virus, mainly from the laboratory point of view, and by Dr. Rees on precautions against tuberculous infection in the animal house and laboratory. Both these speakers were concerned mainly with the techniques which would reduce risks to the workers with the agents concerned and both referred to the advisability of vaccination of staff. Surgeon Commander Darlow dealt with the general topic of the provision of clean air as a factor in preventing the spread of infection in the animal house. He showed how considerable reduction in the number of airborne organisms could be effected by suitable air filtration. The use of ultra-violet light was recommended as the simplest method for killing airborne organisms. Mr. Porter dealt with the traumatic hazards not only from the animals themselves but also from damaged or incorrectly designed equipment. From the economic point of view, the minor accidents of the animal house (graphically illustrated by Mr. Porter) interfere considerably with efficient working. Cracked water bottles, damaged cages and bins all provide sharp edges on which fingers and hands can readily be cut or scraped, with consequent loss in working time.

Summarizing the discussion, the chairman, Dr. J. B. Brooksby, said that the first point that had been made was the necessity for awareness among research workers of the risks involved in handling any species. A remark had been made from the floor that any worker about to begin experiments with monkeys would do well to consult some of the laboratories concerned in handling the species in large numbers. Similarly, any worker dealing with any new or exotic species should try to obtain all possible information before beginning his experimental work. This symposium might, however, serve as an initial stage in the documentation of the various agents which should be considered as hazardous in the animal house. An important point seemed to be

that the various risks were kept in their true perspective. Thus, *B* virus infection from the bite of an apparently healthy monkey was obviously far more serious than the unpleasant, but relatively trivial, infection by ringworm from a domestic animal. Just as the severity of infection varied from agent to agent, so the chances of infection from animal to man would vary, and the proper assessment of the dangers involved in any particular type of work would take into account these factors.

The action to be taken to avoid the hazards of the animal house would obviously relate to the degree of risk. Commander Darlow's paper indicated the way to the ultimate degree of protection possible, by designing a special building with air-filtration and all other apparatus necessary for restriction of airborne micro-organisms. Various practical steps could, however, be taken to reduce risks in accommodation already available. Several speakers emphasized the need for taking only healthy animals into the animal house. The value of tuberculin testing in monkeys was discussed, and there was some divergence of opinion as to its value. Restriction of risk in operative procedures could often be achieved by the use of hoods, anti-splash guards and other equipment of this sort. The point had also been made that spread from an animal often occurred after the object of the experiment had been achieved and there was no case, for example, for keeping tuberculous guinea pigs after the primary lesion had developed; they could be destroyed before caseation supervened. Another problem which was of great importance was staff discipline, particularly where large numbers of animals were being handled and much of the work was necessarily undertaken by staff recruited untrained. Training took place after appointment, when new staff members were undertaking potentially dangerous work. Attention to proper sterilization techniques and to the disposal of bedding and dirty equipment was most important. The use of proper equipment in a good state of repair was also a prime necessity for the avoidance of undue risks.

It will be realized that many of the steps that must be taken to control hazards are also those which would be taken in any animal house dealing with infective agents, in order to prevent cross-infection among the animals themselves, and the instruction of workers in the health hazard may help to achieve the discipline which will, in turn, prevent both the health hazard and the hazard to the experiment itself.

USE OF ANIMAL CELL, TISSUE AND ORGAN CULTURES IN RADIOBIOLOGY

FOR nearly fifty years the tissue culture method has proved a valuable experimental tool for radiation studies. By isolating a group of cells outside the body the effect on blood- and nerve-supply and the reaction of the organism as a whole are eliminated and the direct effects of radiation can be accurately assessed. The establishment of clone cultures and the introduction of chemically defined media in the past decade have brought a further simplification and standardization of experimental conditions which make it possible to obtain precise quantitative data. At a symposium convened by the New York Academy of Sciences in February 1961, the effects of ionizing radiations on cell structure and differentiation, on mitosis and growth of cells in tissues grown *in vitro* were reported and correlated with changes in synthesis of deoxyribonucleic acid (DNA); the influence of oxygen tension, sensitizing and protective agents on radiation action was outlined, and variations in radiosensitivity discussed.

L. J. Tolmach (St. Louis) and J. E. Fogh (New York), using HeLa *S3* and *FL* amnion cells, reconfirmed the well-known fact that radiation affects cell division much more than cell growth. Cells unable to divide continue to increase in size, reaching a maximum 2–4 days following exposure.

Y. Onuki, A. Awa and C. M. Pomerat (Pasadena) investigated the effect of radiation on the chromosomes of leucocytes cultured from the human buffy coat. A dose of 400 r. increased the number of tetraploids by 7 per cent and the number of cells showing chromosomal damage by 50 per cent. F. E. Osgood (Portland) interpreted the effect of radiation on normal and malignant human hæmic cells *in vivo* and *in vitro* in terms of his α-cell–*n*-cell concept. The potentially dividing α-cells give rise, as a rule, to another α-cell and a differentiating *n*-cell. Doses of 10–400 r. immediately inhibit the division of α-cells while the number of fully differentiated and longer-lived *n*-cells remains, at first, unchanged. Their number begins to drop when the reduced α-cells fail to replace *n*-cells which have reached the end of their natural life-span. In this range of dosages, radiation did not seem to inhibit synthesis of DNA of α-cells as they continue to incorporate tritiated thymidine after their increase in number is stopped. Comparisons of hæmic cells as regards their radiosensitivity show that normal and malignant lymphocytes are the most sensitive cells though even in these cells there is a marked individual variation in sensitivity.

The great sensitivity of lymphocytes was also emphasized by Schrek and Trowell. R. Schrek (Hines, Ill.) examined the immediate and delayed effects of radiation on human and animal lymphocytes. A dose of 1,000 r. produced severe intranuclear vacuolization and death within 24 hr. following exposure, while 10 r. reduced the 10 per cent survival-time from 9·7 to 6·1 days. There was no difference in radiosensitivity of human lymphocytes from blood or lymph nodes, but lymphocytes from rats, guinea pigs and rabbits were slightly less sensitive than human lymphocytes. O. A. Trowell (Harwell) described the influence of various gas mixtures on the action of radiation on lymphocytes from rat mesenteric lymph nodes and stressed the importance of choosing the right effect and end-point for measurements. For example, by counting dead nuclei 5 hr. after exposure an oxygen sensitivity factor of 12 was found, while counts of surviving nuclei 24 hr. after irradiation gave a sensitivity factor of 2·7—a value which agrees well with most other biological materials. Trowell attributes his original result to a slowing down of pycnosis by oxygen.

Another aspect of the action of radiation which received much attention was the effect on synthesis of DNA as measured by the uptake of tritiated thymidine and its correlation with mitotic inhibition and cell death. H. Harrington (Cleveland), using *U*12 fibroblasts, reconfirmed the classic findings of Strangeways and Oakeley on the effects of radiation on cell division. A rapid drop in mitosis after 500 r. is followed 13 hr. later by re-appearance of cell division. Counts of thymidine-labelled mitotic figures suggested that there was no accumulation of cells in the *G*1 interphase period but a delay in the synthetic *S* and premitotic *G*2 period, using the terminology introduced by Howard and Pelc. Similar findings were reported by R. B. Painter (Columbus) and W. L. Hughes (Upton), who found no evidence that radiation influenced the rate at which HeLa *S*3 cells moved from the *G*1 to the *S* period. The small immediate inhibition of synthesis of DNA could not be correlated quantitatively with the lethal effect of radiation or was the position of the cells in the synthetic cycle related with the probability of their survival. The three authors agreed that in contrast to synthesis of DNA, synthesis of ribonucleic acid (RNA) was practically undisturbed, even after high doses of radiation.

J. E. Till (Toronto) observed a generalized dose-dependent prolongation of all three stages of the first synthetic cycle following exposure. However, the *G*1 and *S* periods were only slightly prolonged. The greatest effect was seen on cells which had completed synthesis of DNA and had moved to the *G*2 period, which was severely disrupted by radiation. Thus, the effects of radiation on synthesis of DNA seem to be the result, rather than the cause, of mitotic inhibition.

A. N. Stroud and A. M. Brues (Argonne, Ill.) gave an interesting example of differences in synthesis of DNA response in two strains of pig- and monkey-kidney cells. After exposure the monkey-kidney cells which normally have a low rate of thymidine incorporation showed an increase of 15–20 per cent more than that of the unirradiated controls, while the pig-kidney cells which normally have a high rate of incorporation show a striking decrease. P. F. Sanders, D. F. Petersen and W. L. Langham (Los Alamos) described methods for growing HeLa cell suspensions in the presence of tritiated thymidine and tritium water, for determining cell size and cell number and for measuring radioactivity. Beta radiation from tritium has a much greater inhibitory effect on proliferation when emitted from incorporated ^{3}H-thymidine in the nucleus than when merely present in water.

W. B. Looney (Baltimore) pointed out some limitations of labelled compounds in radiobiological studies. Erroneous interpretations may be made as a result of reduced synthesis of DNA unless differences in time-sequence of control and irradiated systems are taken into account. For example, the generation time of hepatocytes in regenerating rat liver is increased from 8 to 13 hr. after 3,000 r.; thus, the ratios of synthesis of DNA of irradiated and control specimens between 8 and 13 hr. would be the ratio of the activity in a second cycle of hepatocytes in the controls to that of the first cycle of irradiated hepatocytes.

T. R. Munro (Cambridge) described a technique which allows selected parts of single cells to be irradiated with α-particles and showed a film of irradiated chick fibroblasts. When the chromosomes were irradiated with a sufficient dose sticky bridges were seen at anaphase and the reconstruction of daughter nuclei inhibited. Neither of these effects was seen if the radiation was confined to the cytoplasm. He also described a method which allows single HeLa *S*3 cells and their progeny to be followed for a week. This technique should enable cell survival to be studied after localized radiation.

G. O. Gey (Baltimore) described a more elaborate analogous technique for studying population dynamics of lymphoblasts in culture. His apparatus photographed a number of accurately defined fields of cells at intervals of 15 min. and enabled the fates of large numbers of single cells to be studied.

H. B. Hewitt (London), using an *in vivo* bio-assay of mouse leukæmic cells, measured their response to radiation and its variation with different degrees of oxygenation. For whole-body exposure of mice breathing air or oxygen the dose producing the same survival curves of leukæmic cells was lower by a factor of 2·3 as compared with mice killed shortly before irradiation. On the other hand, survival curves for cells of a solid sarcoma were similar for living and recently killed mice and identical with the anoxic curve obtained for the leukæmic cells. He concluded that variations in radiosensitivity between different mouse tumours are due to differences in their oxygenation. J. R. Andrews and R. T. Berry (Bethesda), using the same technique, gave the survival curves of a different strain of mouse leukæmic cells which grow normally under anoxic conditions in the animal. The cells showed an oxygen factor similar to that found by Hewitt. They also reported preliminary results on the effect of dose fractionation and on sensitization by some pharmacological agents.

A. Goldfeder (New York), in an interesting paper, gave her results on electron-microscopic and biochemical studies of tumour mitochondria, and showed that variations in radiosensitivity of two types of mouse tumour cells could be correlated with differences in mitochondrial structure and metabolism.

R. Bases (New York) demonstrated the sensitizing action of actinomycin *D* and deuterium oxide in experiments in which Puck's single-cell technique was employed. A dose of 300 r. sterilized 5–10 times as many cells pretreated with actinomycin *D* than controls. Cells pretreated with deuterium oxide were also abnormally sensitive to radiation, an effect attributed to a presensitization by incorporation of the drug into the DNA of the cells.

D. E. Rounds (Pasadena) reported a protective effect of yeast RNA. RNA-treated cells of the Fernandes strain of amnion cells multiplied at a normal rate following radiation while untreated cultures showed immediate inhibition, but a minimum time of 8 hr. was necessary before protection could be seen. The magnitude of the protection varied considerably with different cell strains. A. Schleich (Heidelberg) reported her results on radioprotective properties of various compounds, among them histamine, thioctic acid, cysteine and cysteinamine, tested on whole-body irradiated mice, a strain of human malignant fibroblasts and solutions of thymine and DNA. The protection varied with the system tested but was consistent for all systems with cysteine and cysteinamine.

In contrast to the previous papers, which dealt mainly with the effects of radiation on established cell strains, J. G. Carlson, E. Borghese and I. Lasnitzki described the action of radiation on freshly explanted cultures of organized tissues, which retain their histological structure *in vitro*.

J. B. Carlson (Knoxville) pointed out the advantages of his grasshopper neuroblast technique for quantitative radiation studies. The large size of the neuroblasts, their position on the surface of the embryo, their constant number and fixed arrangement in relation to each other simplifies cell counts, facilitates their identification and allows radiation effects to be followed through several division cycles. A disadvantage of the method is the great sensitivity of cell division to changes in osmotic pressure.

As in vertebrate tissues, X-radiation produces a mitotic inhibition due to an arrest of cells in late prophase followed by mitotic recovery. Increasing the oxygen tension does not increase mitotic inhibition but reducing it diminishes the effect of radiation. Investigations of frequencies of chromosome aberrations induced by X-rays show a peak at middle prophase and anaphase.

E. Borghese (Rome) showed in a beautiful film the growth of mouse embryonic lung explanted by the organ culture technique and the effects of γ-rays on this tissue. In the controls, development and outgrowth of clear translucent bronchi could be observed. After exposure the explants became opaque, growth slowed down and finally stopped.

I. Lasnitzki (Cambridge) reported that irradiation can change the pattern of differentiation in organized tissues grown *in vitro*. Mouse vaginal explants grown by the organ culture technique form squamous epithelium with a superficial layer of keratin. Exposure to X-rays inhibits the squamous development and keratin formation both in normal- and œstrone-treated vaginas. This effect is unrelated to mitotic inhibition and closely resembles that seen after treatment with excess vitamin A. It also agrees well with results obtained in *in vivo* experiments.

The symposium reflected the present trend to measure the biological effects of radiation and showed the advantages tissue cultures offer for such work.

Ilse Lasnitzki

THE NATIONAL CHEMICAL LABORATORY

THE report of the National Chemical Laboratory, 1960*, comprises the report of the Steering Committee over the signature of Prof. C. E. H. Bawn, and the report of the Director, to which are appended details of membership of the sub-committees of the Steering Committee and of the staff of the Laboratory at December 31, 1960, and lists of publications and patents during the year and of Standard Samples of Organic Compounds.

On consideration of the Laboratory's research programme in greater detail, the Steering Committee agreed that the work of the Extraction of Metals Group should include fundamental work on the inorganic and physical chemistry of hydrometallurgy in addition to work for the Atomic Energy Authority on ores and minerals. While the Radioisotope Applications Unit, established at Harwell as an out-station of the Laboratory in 1957 and now at the Isotope Research Division of the Atomic Energy Research Establishment at Wantage, is regarded as contributing to the National Chemical Laboratory's mandate to cultivate instrumental techniques, the Steering Committee found no justification for the Department of Scientific and Industrial Research to undertake any work on the direct uses of high-activity sources of radiation.

Considering the building programme of the Laboratory in relation the future development of its research programme, the Steering Committee formed the opinion that, to cover the possible forward requirements, the present staff of 264 should be increased by about one-half in the next 5–7 years and doubled in the next 10–15 years. It anticipates that the need for Government research in the chemical field will inevitably grow with the continued expansion of the chemical industry, and to provide the buildings needed for a staff more than double the present complement it recommends that the Laboratory be moved to a larger site as near the present site as possible, in view of the close and important co-operation with the National Physical Laboratory. The site should be large enough not only to accommodate the buildings for the National Chemical Laboratory but also to provide in the long term for possible expansion there of other existing laboratories or for new laboratories. The Research Council has already agreed that the possibility of acquiring such a site should be explored.

Commenting on the problem of determining the best balance of effort in a national laboratory, the director, Dr. J. S. Anderson, defines the task of the National Chemical Laboratory as that of carrying out research in areas that are important but that cannot be studied so effectively by agencies less centrally placed. It should seek to place new developments on a firm basis of scientific understanding, to ensure that all its investigations enrich scientific knowledge, and to bring a deeply analytical approach to bear on those major problems of wide application, such as the prevention of corrosion, which fall within its scope. The present research programme is still being developed, and Dr. Anderson notes some material changes in the work of several research groups during the year. Work on the physicochemical properties of organic compounds has been converted into a much more comprehensive programme of chemical thermodynamics, though full effect cannot be given to the projected scheme until the new buildings are available. The Extraction of Metals Group, which hitherto has been working almost entirely in associations with, and at the request of, the Atomic Energy Authority, is now including in its programme some fundamental work on the more familiar metals of industry. Collaborative arrangements with the National Research Development Corporation for work on the development and application of ion-exchange membranes and of gold-selective ion-exchange resins was terminated, but some work in this field is being pursued at a more fundamental level. Work in the Inorganic Group on the extreme

* Department of Scientific and Industrial Research. *Report of the National Chemical Laboratory, 1960: Report of the Steering Committee and Report of the Director of the National Chemical Laboratory for 1960.* Pp. iv + 70 + 4 plates. (London: H.M. Stationery Office, 1961.) 4*s.* 6*d.* net.

purification of semiconductor materials was also substantially reduced and effort transferred to applications for the substantial quantities of rare-earth elements that are becoming available.

A small research unit was set up to continue the Director's fundamental research on the chemistry of solids, and in this unit the study of physical processes and chemical reactions on absolutely clean metallic surfaces, using the field-emission microscope, has commenced. A research fellowship has been established and work started on the related and very powerful technique of ion microscopy, which is capable of imaging individual atoms on the metal surface. The Chemical Thermodynamics Group made a critical examination of existing data, and, with the assistance of the Association of British Chemical Manufacturers, this has led to a recommendation that, on the organic side, the Laboratory should commence work on the aliphatic compounds containing oxygen and continue its work on phenols. The study of the purification and physical properties of phenol, cresols and xylinols has been completed for publication; work continued on the precise determination of the critical temperatures of certain pure hydrocarbons and the ebulliometric apparatus for measuring vapour pressure has been re-designed and re-built. In the Non-metals Group, particular emphasis was placed on boron–nitrogen compounds, and the systematic study of the chemistry of borazole and its derivatives promises to provide polymers with greater thermal stability than is usually associated with a molecular skeleton of carbon–carbon bonds. Compounds of promise as pre-ignition suppressors for motor 'petrol' have been obtained. Work on ion exchange materials, particularly ion-exchanging membranes, has been narrowed to a thorough study of selectivity and its exploitation, and increased attention given to polymers with potentially applicable electrochemical and electrical properties in the form of reversible redox polymers and high nolecular weight materials likely to exhibit semiconduction.

The Extraction of Metals Group has developed a modified rotary-film disk contactor for the solvent extraction of metals direct from solutions containing appreciable quantities of solids in which solvent and pulp flow parallel to the planes of the disks, and each stage contains several disks. This has given excellent recoveries of uranium, with acceptable solvent losses, from sulphuric acid-leached ore pulps and sulphuric or nitric acid-leached uranium concentrates. Promising results were also obtained in extraction of uranium by heap leaching with acid, and in co-operation with the Warren Spring Laboratory, the extraction and concentration of beryllium from concentrates containing 2–4 per cent beryllium oxide, derived by flotation from low-grade ores, has also been studied. Examination of the solution chemistry of beryllium, mainly concerned with developing methods for the selective recovery of beryllium from sulphate leach liquors, showed the pronounced tendency of beryllium to form polynuclear species in both weakly acidic and alkaline solutions.

The Inorganic Group has given considerable attention to the extraction of the reducible elements, samarium, europium and ytterbium, by sodium amalgam, and has developed a continuous process for recovering these elements from concentrates. The possibilities of a new and rapid method of extracting aqueous solutions of lanthanons with tributyl phosphate are being examined and the purification of gallium and arsenic is being studied with the view of the preparation of gallium arsenide as a semiconductor. The Corrosion of Metals Group is seeking a better understanding of the basic physico-chemical factors which control corrosion and oxidation processes, and of which account must be taken in devising solutions to specific problems. Special attention has been given to the relative importance of the surface preparation of mild steel and the anions present in the solution in which the steel is immersed. In the study of corrosion in moving water, attention has been given to the chemical nature of the corrosive products. The very high initial corrosion-rate of mild steel in oxygenated dilute chloride solutions at 200° C. has been determined, and relations have been established between the rate of corrosion of mild steel in batch cultures and the hydrogenase activity of sulphate-reducing bacteria. Work is also in progress on the distribution in soils of different species of sulphate-reducing bacteria, as well as a pilot investigation of the corrosion of mild steel exposed at two sites on the River Thames.

THE NATIONAL RESEARCH DEVELOPMENT CORPORATION

IN its eleventh year of life, the National Research Development Corporation * negotiated more licence and option agreements with United Kingdom and overseas firms than in any previous year. Eighty-one such agreements were completed (as against sixty-one in the previous year) and, by June 30, 1960, some 456 such agreements were in force. Royalties and other payments received under licence and option agreements amounted to £259,000 as against £182,000 in the previous year. About half these receipts derived from overseas business, that is, from direct payments made by manufacturers abroad, and from royalties on goods exported abroad by United Kingdom manufacturers.

The outright sale of patent rights is another, but exceptional, form of exploitation activity, and is a fluctuating source of income.

By the end of 1960 the Corporation had a portfolio of 1,066 United Kingdom and 2,042 overseas patents and patent applications for inventions, derived chiefly from publicly supported research in the Government research establishments, research councils and the universities. The portfolio is under constant change. Unprofitable patents are allowed to expire, others lapse at the end of their periods of grant, and new accessions are continually being included.

At the end of 1960, the Corporation was providing financial assistance for thirty-five development projects. During the year, the Corporation's expenditure on development amounted to £427,000, and with its subsidiaries it has entered into commitments for £739,000 for development purposes. Recoveries of development expenditure amounted to £52,000.

* *National Research Development Corporation Bulletin*, April 1961.

XUM

One project which was commenced in the year under review was the automatic process plant control. This is concerned with the development of electronic automatic control equipment suitable for plant and process control automation and, for example, for the control of power stations and chemical plants. It contemplates the development of a range of standard equipment which will permit electronic analogue and mixed analogue/digital computing techniques to be used for this purpose, the individual elements being capable of assembly in a variety of ways to enable control systems to be designed as required for any particular plant.

The work is being undertaken by an established firm in the electronic computing and control engineering fields, which is sharing the cost with the Corporation. The project is still in its early stages, but work has started on the design of circuit elements and components, and on the study of potential plant applications.

FŒTAL AND NEONATAL PHYSIOLOGY

ALTHOUGH there have been great reductions in infantile mortality within the present century, it is only now being realized how much can be done to reduce mortality before birth and in the first days after it. The application of fundamental physiological principles to the management of premature infants has become a study in its own right. These principles were recently examined in the *British Medical Bulletin**, the contributions varying from investigations of blood pressures in new-born babies, the acquirement of immunity and immunological tolerance, protein and amino-acid movements in the fœtus and new-born animal, transport mechanisms in the placenta and red cells, enzyme development, the newer aspects of placental histology, fœtal endocrinology, and the chemoreceptors and their function in the aortic-pulmonary body.

Among the more outstanding experimental work is a review by Dr. R. Deanesley of the present position in fœtal endocrinology. Experimental operations by Jost, Wells and their collaborators, on pregnant rabbits and rats, and on fœtuses *in utero*, have shown that in these animals the pituitary and testes become active before birth and play a decisive part in differentiation. Thyroid and adrenal development is controlled by the fœtal pituitary. The developing fœtus at certain stages can react to exogenous hormones which reach it directly or through the placenta. The fact that the endocrine glands are active in the fœtus carries no implication that their secretions can affect the maternal physiology.

In their review of the fœtal kidney, Miss Pauline Alexander and Dr. D. A. Nixon show that development of function in the metanephros is spread over a period of time—some activities appearing earlier than others.

The onset of glomerular filtration precedes tubular activity so that large volumes of urine may be passed into the amniotic or allantoic sacs from an early age. Subsequent appearance of tubular activities results first in the production of a hypotonic and, later, a hypertonic urine. The maximal osmolarity of the urine is low by adult standards because of the low excretion of solute. The renal capacity to acidify the urine is limited in the early stages, in part by the very low excretion of phosphate. The glomerular filtration-rate appears to be somewhat labile at first, and the factors leading to its stability in later life are not known. Ratios for the clearance of *p*-aminohippurate: inulin suggest that the supply of blood to the kidney is low in fœtal life in comparison with that in the adult, and this may limit its functional capacity considerably.

The stage of metanephric development at which a fœtus of any particular species attains an independent existence will determine its initial renal problems.

Dr. Heather J. Shelley has investigated reserves of glycogen and their changes at birth and in anoxia. However, there is no reliable information on the glycogen reserves of the new-born human infants, but, by analogy with other species, they probably have a high concentration of liver glycogen and a fair amount of muscle glycogen. New-born babies have been known to withstand anoxia for up to 30 min. (Bullough, 1958), so it is likely that their cardiac glycogen concentration is also high. Since lactation is not established in man until 24–48 hr. after parturition, the new-born baby must be particularly dependent on its reserves; if its liver glycogen falls as rapidly as in other species it will be in a precarious metabolic state for several days. If kept warm, the new-born baby can survive for several days without food, but if exposed to cold it later dies of hypoglycæmia (Mann and Elliot, 1957), probably the result of an attempt to maintain its body temperature (Bruck, 1959). It is therefore possible that factors which reduce the glycogen content of the fœtus *in utero* or at birth may jeopardize the infant's chances of survival after birth, and future work must determine to what extent the deleterious effects of maternal nutrition, placental insufficiency and birth asphyxia may be the consequence of inadequate glycogen reserves.

The ability of young mammals to withstand a total lack of oxygen was considered by Dr. Joan Mott. She believes that the practical conclusion to be drawn from the present state of knowledge of the conditions under which anoxic survival is possible is that the carbohydrate stores, particularly of the heart, should be protected from dissipation. Resynthesis of carbohydrate does occur, but may be limited by the rate of entry of glucose into the cell. Thus the factor of vital importance is the store of carbohydrate already available within the cardiac cells. Repeated episodes of anoxia or hypoxia are cumulatively as well as individually dangerous, and it is probable that, if the *p*H is not restored to normal level, a stage is reached at which glycolysis may be completely inhibited. Repeated anoxic episodes may also exhaust the liver glycogen and lead to hypoglycæmia, so that maintenance of the circulation might be insufficient to protect the brain from injury.

* *British Medical Bulletin*, **17**, No. 2 (May 1961): Fœtal and Neonatal Physiology. Pp. 79–176+8 plates. (London: British Council, 1961.) 20*s*.

From the available evidence showing the reaction of the new-born animal to environmental temperatures, Dr. June Hill suggests that the metabolism of the new-born of a warm-blooded species often differs from that of the adult animal in two important respects: first, the new-born animal has a lower basic metabolic rate than is appropriate for its surface area; and secondly, per unit surface area, the new-born animal has a smaller maximal metabolic response to cold. Thus the heat production of the new-born animal, both minimal and maximal, is lower per unit surface area than that of the adult. The degree of immaturity at birth probably determines to what extent the metabolism of the new-born deviates from the adult pattern.

In addition, the new-born animal often has a coat of lower effective insulating power than that of its parent; this is partly because the new-born is smaller than the adult, and is partly a specific attribute of immaturity. This means that, per unit surface area at a given environmental temperature, the new-born animal loses heat much more readily than the adult.

These facts underlie, and go far to account for, the difficulties which many new-born animals experience in maintaining a stable internal body-temperature.

LIFE AND SCIENTIFIC WORK OF SIR JAMES HALL

THE Bruce-Preller Lecture of the Royal Society of Edinburgh was delivered on June 5 by Dr. V. A. Eyles, formerly of the Geological Survey of Great Britain, who took as his subject, "The Life and Scientific Work of Sir James Hall, Bart., F.R.S. (1761–1832), Second President of the Royal Society of Edinburgh".

Sir James Hall, fourth Baronet of Dunglass, Haddingtonshire, was born on January 17, 1761. He was a great nephew of Sir John Pringle, Bart., the distinguished Army physician, a president of the Royal Society of London. Owing to the early death of Hall's father, Sir John took charge of his education. He attended the Universities of Cambridge, Geneva and Edinburgh. After extensive travel in Europe he settled down at Dunglass, though spending much time at his town house in Edinburgh. In 1786 he married Lady Helen Douglas, a daughter of the Earl of Selkirk.

Hall had been elected a member of the newly formed Royal Society of Edinburgh in 1784, and for many years took a prominent part in the affairs of the Society. He became president in 1812, being the first scientist to occupy the presidential chair. He was elected Fellow of the Royal Society of London in 1806.

Though chiefly remembered as a geologist of the Huttonian school, he was always much interested in chemistry, having been a student of Joseph Black, in Edinburgh. His first address to the Royal Society of Edinburgh was on Lavoisier's new theory of chemistry, to which he had been converted by Lavoisier himself, whom he had met a number of times in Paris.

Hall contributed a number of papers to the Edinburgh *Transactions*, chief among them being his "Account of the Effects of Compression in Modifying the Effects of Heat". This describes the long series of experiments by which he demonstrated that limestone, when heated under pressure, remains undecomposed. This paper has become a classic, and illustrates Hall's great skill as an experimentalist. Hall has been called the 'father of experimental geology', but might, perhaps more aptly, be called the 'father of geochemistry'. His skill as an experimentalist has tended to obscure the fact that he was also an extremely able field-geologist.

Hall was a man of many interests, including, in later life, politics, and he was member of Parliament for the St. Michael division in Cornwall during 1807–1812. He died in 1832, after suffering ill-health in his later years.

HOSPITAL IN-PATIENTS IN BRITAIN

A REPORT on the Hospital In-Patient Enquiry for 1959 gives provisional figures for that year and some comparisons with earlier years*. The report is based on data provided by hospitals (other than psychiatric hospitals) in the National Health Service, on forms giving a confidential summary of the case records of a 10 per cent sample of discharges (the term includes deaths). The purpose of the inquiry is to provide statistics to assist the administration of the hospital service and to supplement other statistics about mortality and illness in the community. It is organized jointly by the Ministry of Health and the Central Register Office. 1959 was the second year in which the inquiry had almost complete coverage of the relevant hospitals in England and Wales.

The tables in the report show the diagnostic distribution of case discharges in 1959 and in each year back to 1955, regional figures for selected important conditions back to 1956, regional figures for maternity cases according to the type of maternity care received, and the distribution of the cases in the 1959 sample by sex, age and diagnosis. It also includes the estimates of the population of hospital regions by sex and age at June 30, 1959.

There were, in all, 3,664,100 discharges from the relevant hospitals in 1959, or 807·3 per 10,000 population, compared with 3,554,700, or 788·0 per 10,000 in 1958, an increase of 2·4 per cent. The estimated numbers of discharges for most common causes of admission to hospital showed some increase, though much of this was probably due to more efficient use of hospital services rather than to increased illness.

Respiratory tuberculosis continued to decrease. There were 44,300 discharges in 1959, 9·8 per 10,000 population, compared with 48,800 and a rate of 10·8 in 1958. The rates for males fell in all regions except Liverpool, where an intensive mass radiography campaign was held early in the year.

* Ministry of Health and General Register Office. Report on Hospital In-Patient Enquiry for the year 1959. Part 1: Preliminary Tables. Pp. 16. (London: H.M. Stationery Office, 1961.) 1s. 6d. net.

XUM

There were 2,200 cases of acute poliomyelitis discharged, or 0·5 per 10,000 population, 1,000 fewer than in 1958; there has also been a steady rise in hospital discharges for cancer of the lung, from 22,400, 5·0 per 10,000 population, in 1955, to 30,600, 6·7 per 10,000, in 1959. Discharges for other tumours benign and malignant, have remained fairly steady.

Discharges for coronary disease continued to rise. The 1959 figure was 56,600 (12·5 per 10,000 population), 6,800 more than in 1958. For other heart diseases and hypertensive disease, combined, there were 5,500 fewer discharges in 1959 than 1958. 44,600 cases of varicose veins were discharged, 9·8 per 10,000 population, compared with 40,900 and 9·1 in 1958.

Figures for influenza, bronchitis and pneumonia all showed an increase over 1958, but whereas hospital cases of influenza fluctuate (2,600 in 1955 and 1956, 22,200 in 1957, 4,200 in 1958 and 10,000 in 1959), pneumonia and bronchitis have increased steadily from 58,000 and 35,400, respectively, in 1955 to 81,000 and 60,200 in 1959.

In the two years since 1957, discharges for head injuries increased by 20 per cent to 82,300, a rate of 18·1 per 10,000 population, and other injuries by 13 per cent to 219,200 and a rate of 48·3.

The number of maternity discharges in 1959 was 612,400, being 80·2 per 100 births, compared with 599,000 and 75·9 in 1958. Of all births, 60·5 per cent took place in hospital compared with 57·5 in 1958.

ORIGINS OF PETROLEUM

SIR ROBERT ROBINSON has put forward an intriguing new theory suggesting a duplex origin of mineral oils.* The prevailing view is that biologically derived organic matter aged and was transformed into petroleum; but the organic chemistry of this supposed petroleum has never been satisfactorily worked out.

In place of this conception, Sir Robert suggests a duplex mixture which, wherever it was situated, had plenty of time to find its way to the littoral regions of the oceans and there to become admixed with organic life. As in the full biological theory, life would be promoted by salts brought down by the rivers.

It was indicated by Oparin and Urey that the primeval atmosphere was probably largely methane, carbon dioxide and ammonia and the temperatures rather high. The atmosphere of Venus may be similar. It contains much carbon dioxide, and Hoyle has suggested that the clouds could consist of oil. Organic matter has been found in meteorites. Loss of hydrogen from methane would yield hydrocarbons and the thermodynamic equilibrium would be disturbed by escape of hydrogen from the atmosphere. Urey and Miller have shown that simple amino-acids, for example glycine and aspartic acid, can be produced in the laboratory naturally, by irradiation of such a mixture after cooling. Quite recently, Bahadur and Srivastava have made peptide from the same amino-acids by photosynthesis in aqueous solutions. The simple, early non-chlorophyllic organisms might well have used hydrocarbons as a source of energy, and possibly become covered with oil concentrated near the shores. It is hard to see that the distribution of oil which is now observed presents any insuperable obstacle. Almost all geochemical distributions are curiously uneven. The primeval oil may have been on the surface of the seas and brought to shore by solids such as small organisms and rock dust combined with the action of the tides. The release from miscelles, as suggested by Baker, is another possibility.

There are several mechanisms for straight-chain synthesis. Among these the most likely are the Fischer-Tropsch and oxo-synthesis. If olefines were present, or developed from products of oxo-synthesis, they could react with carbene to alkyl*cyclo*-propanes. The latter are known to give straight-chain α-olefines on pyrolysis as there is ample room for isomerization, branch-chain synthesis, aromatization and other cyclization, introduction of nitrogen and sulphur and so on in varied and inevitable side reactions.

* *Catalyst*, 6; Spring, 1961.

NEW AFTERGLOW EMISSION ASSOCIATED WITH METASTABLE NITROGEN ATOMS

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ATOMIC two-body recombination is of importance if the atomic concentration is small enough. This is known to occur in interstellar space, for example. In laboratory experiments three-body recombination is usually the most important. When the gas pressure is small, the atoms diffuse towards the wall where they are de-activated before they recombine by two-body collisions. Addition of an inert gas reduces the diffusion effect. One may then observe strong emissions of atomic lines of forbidden transitions or atomic recombination spectra. These emissions are usually characterized by a strong afterglow since the recombination processes are slow or the life-times for the forbidden transitions very large, of the order of seconds. In the present article a new example is given of atomic recombination involving excited nitrogen atoms.

The discharge tube is a 1-l. glass bulb with two side-tubes sealed in. One contains a 2-mm. diameter tungsten rod which may be used as an internal electrode. The second tube contains phosphorus pentoxide and sodium trinitride powder. Phosphorus pentoxide eliminates the last traces of water vapour; sodium trinitride is used to produce small quantities of pure nitrogen.

In some experiments, in order to improve and accelerate the purification of nitrogen, a portion of the inner surface of the glass bulb has been coated with a thin layer of solid sodium. After careful evacuation, the glass bulb is filled with pure xenon

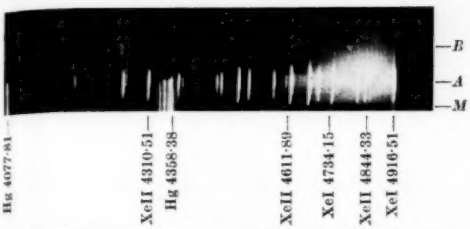

Fig. 1. Afterglow of a nitrogen–xenon mixture. *A*, high frequency discharge spectrum showing the XeI lines and the semi-continuous band system; *B*, short duration afterglow emission spectrum; *M*, mercury comparison spectrum

gas at a pressure of 20 mm. mercury and sealed off. The discharge and the afterglow spectra have been recorded using a conventional pulse technique and a rotating disk. The high-frequency generator had a pulse frequency of 50 cycles per sec. and a pulse duration of 10 μsec. The high frequency–high voltage output was applied directly to the tungsten electrode of the glass bulb.

The rotating disk was driven by a synchronous electric motor having the same frequency as the pulse generator. It is possible to obtain either the discharge or the afterglow spectrum, at the selected time delay, using the phase-shift circuit of the generator. Often it is interesting to obtain simultaneously the discharge and the early afterglow spectra. This can be achieved if the edge of the slit of the rotating disk is perpendicular to the slit of the spectrograph, and has been done for the spectrum shown in Fig. 1. Such a comparison is often useful: it is then quite certain that the experimental conditions are exactly the same for both the discharge and the afterglow spectra.

Fig. 1 shows a print of the spectrum obtained with the pulse generator, the rotating disk, and a glass spectrograph of *f*/8 aperture. The exposure time was 3 hr. This spectrum is obtained with a single exposure in which half the slit admitted light from the discharge (during the pulse of the generator), and the other half of the slit the early afterglow emission. The discharge spectrum *A* shows most of the XeI lines located in the blue and in the violet (the XeII lines in the red are not recorded in this spectrum). The remarkable feature is that the afterglow emission does not seem to be much excited in the discharge. The afterglow spectrum shows a vibrational structure which seems to merge into a continuum near 4790 Å. This continuous emission extends to about 4600 Å.

The experimental fact gleaned from Fig. 1 is that the semi-continuous band system has about the same intensity in the discharge as in the afterglow. It suggests strongly that the emission is associated with a slow reaction. It is emitted only if nitrogen is present. The emitting molecule is very likely diatomic. Any polyatomic molecule would be dissociated in the discharge in a matter of seconds. Since xenon and nitrogen are both necessary for the emission, the diatomic molecule is either an unstable XeN or one of the weakly bound excited nitrogen molecules.

Other semi-continuous spectra which also seem related to atomic forbidden transitions have been found in mixtures of xenon and oxygen. For example, there is a very strong green emission related to the forbidden 5577·35 Å. ($2p^4\ ^1D - 2p^4\ ^1S$) line. Contrary to the emission of Xe + N_2 investigated here, the 5577·35 Å. oxygen line lies at the limit of the semi-continuous emission. It corresponds to the asymptotic portions of the upper and lower molecular potential curves. In the present case (Xe + N_2) the wave-length limit should be at 5200 Å., corresponding to the forbidden transition of atomic nitrogen $2p^3\ ^4S^0 - 2p^3\ ^2D^0$. Actually, the short wave-length limit is about 4920 Å. This differs from the forbidden nitrogen line by 1,383 cm.$^{-1}$ = 0·171 eV.

There is, however, a large difference in the life-times for these two lines. The transition probability for the oxygen green line is 1 sec.$^{-1}$ while that of the nitrogen λ 5200 Å. line is 8×10^{-5} sec.$^{-1}$, about 10^4 times smaller. This difference may be the reason why the 5200 Å. line is not observed in the laboratory.

The emission may be due to recombination of two metastable or normal nitrogen atoms. The lowest states of neutral nitrogen are:

$$N(2p^3\ ^4S^0),\ N(2p^3\ ^2D^0) \text{ and } N(2p^3\ ^2P^0)$$

having the energies:

0·00 eV., 2·37 eV. and 3·56 eV.

The possible recombination processes are:

$$M + N(^4S) \rightarrow MN(S)$$
$$M + N(^2D) \rightarrow MN(D)$$
$$M + N(^2P) \rightarrow MN(P)$$

where M is a nitrogen or xenon atom. The letters S, P and D in the right side next to MN indicate molecules built up with nitrogen S, D and P atoms. As is customary, we shall assume that the pairs of colliding atoms follow a very shallow potential energy curve.

If M is a nitrogen atom, the shallow potential energy curves will intersect other potential energy curves of stable molecular states, in the region of internuclear distances $r > r_e$.

For $N(^4S) + N(^4S)$, for example, intersections occur with $B^3\Pi_g$ at $v \geqslant 12$, $Y^3\Sigma_u^-$ at $v > 7$, $\alpha'\ ^1\Sigma_u^-$ at $v > 6$, $a^1\Pi_g$ at $v > 6$, . . . For $N(^4S) + N(^2D)$, an intersection occurs with $C^3\Pi_u$ at $v > 3$.

However, there should be a chance for direct transitions between the unstable repulsive potential curves. The spectra emitted will be located in the following wave-length regions:

$N(^4S) + N(^2D) \rightarrow N(^4S) + N(^4S) + h\nu$		
12·139 eV.	9·756 eV.	2·383 eV.
$N(^4S) + N(^2P) \rightarrow N(^4S) + N(^4S) + h\nu$		
13·331 eV.	9·756 eV.	3·575 eV.
$N(^4S) + N(^2P) \rightarrow N(^4S) + N(^2D) + h\nu$		
13·331 eV.	12·139 eV.	1·192 eV.
$N(^2D) + N(^2D) \rightarrow N(^4S) + N(^4S) + h\nu$		
14·522 eV.	9·756 eV.	4·766 eV.
$N(^2D) + N(^2D) \rightarrow N(^4S) + N(^2D) + h\nu$		
14·522 eV.	12·139 eV.	2·383 eV.
$N(^2D) + N(^2D) \rightarrow N(^4S) + N(^2P) + h\nu$		
14·522 eV.	13·331 eV.	1·191 eV.

In these equations it is assumed that the emission takes place while the atoms are on the horizontal asymptotic part of the potential curve. If transitions from the shallow minima of the unstable potential curves are considered, slightly different wave-lengths may be obtained. This would explain the difference of 1,383 cm.$^{-1}$ discussed above.

Then our afterglow spectrum would correspond either to the first or the fifth process. The first reaction is more likely since the number of $N(^2D)$ atoms is much smaller than that of $N(^4S)$ atoms.

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CHEMISORPTION AT SOLID-LIQUID INTERFACES

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ADSORPTION of solute molecules or ions from aqueous solutions at a solid surface may result in strong electron interaction between one or more atoms of the adsorbed material and the solid. In such cases water molecules must be displaced and if the adsorption sites correspond to close proximity of metal ions in the solid lattice the adsorption process is formally similar to the first stage in the formation of a co-ordination complex compound. For a simple hydrated metal ion M^{n+} and ligand L, assumed here to be uncharged, this is represented by the following reaction with equilibrium constant K_1:

$$[M(H_2O)_x]^{n+} + L \rightleftharpoons [M(H_2O)_{x-y}\,L]^{n+}$$

If this model of adsorption is applicable there should be a correlation between the extent of adsorption and the value of K_1 for an appropriate equilibrium in solution. Since the environment of a metal ion controls its electron energy-levels, direct comparison is impossible, but by use of a simple argument in the interpretation of some adsorption isotherms it is shown below that the model predicts the observed behaviour.

If certain transition metal ions are placed in order of increasing values of K_1 with a ligand which gives metal-oxygen or metal-nitrogen bonds, it is found that the order is the same for many different ligands (the Irving-Williams series). The values of log K_1 for complex formation with ammonia, extrapolated to infinite dilution, at 30° C. are [1] for Cu(II), 4·01; Ni(II), 2·67; and Zn(II), 2·18. It may be confidently predicted, therefore, that with primary aliphatic amines copper will form more stable complexes than will the other two metals, and the complexes of nickel will be slightly more stable than those of zinc. Thus such an amine should be adsorbed more strongly from an aqueous solution by copper oxide than by nickel oxide and more strongly by this than by zinc oxide, if the arguments presented above are correct.

Adsorption of *n*-dodecylamine on quartz occurs from solutions of *p*H around 9 by attachment of alkylammonium ions to the solid[2,3]. At higher amine concentrations multilayer formation occurs by abstraction of uncharged amine molecules. The results presented in Fig. 1 show that cupric, nickel and zinc oxides adsorb amine molecules, not the ions. The curves show the variation with *p*H of the minimum concentration of total amine (molecules and ions) in solution capable of giving 'pick up' of solid particles of the oxide of size such that they pass through a sieve with openings 0·211 mm. but not through one with 0·152 mm. openings. 'Pick up' is said to occur when the particles under the amine solution adhere so firmly to an air bubble that they do not readily break away when the bubble is raised through the liquid. For particles of similar size and mass it is generally agreed that pick up occurs when the surface coverage with a particular reagent reaches a certain value which depends on the system. The pick up curves for the three metal oxides correspond to lines of constant free-amine concentration in the solution. The analogous curve for quartz corresponds to constant alkylammonium ion concentration in solution. The lack of adsorption of the ions by the metal oxides from solutions of low amine concentration is probably associated with the much higher density of negative charge at the surface of quartz in solutions of *p*H 9–10.

In Fig. 2 adsorption isotherms measured at 30° C are given for *n*-dodecylamine on cupric, nickel and zinc oxides. A full discussion of these will be presented elsewhere. In the case of cupric oxide a well-defined flattening of the curve corresponds to completion of a monolayer, and at higher amine concentrations multilayer formation occurs. Zinc and nickel oxides do not show completion of the monolayer but analysis of the curves indicates multilayer formation in the same region of amine concentration in solution as gives multilayers on cupric oxide. While the monolayer is being formed it is clear that the ability to adsorb amine from solution increases in the order ZnO<NiO<CuO.

The entropy changes on adsorption of amine to the metal oxides before completion of the monolayer will be of similar magnitude in each case. The enthalpy changes will, therefore, also be in the order CuO>NiO>ZnO as will the strengths of the bonds between the metal ions and the amine adsorbed relative to those between the metal ions and water. The strength of the bond between a metal ion and

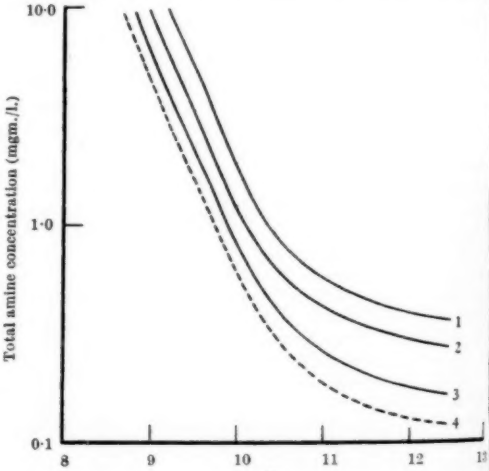

Fig. 1. Bubble pick-up curves for *n*-dodecylamine and (1) ZnO, (2) NiO, (3) CuO. Curve (4) shows the slope of lines of constant free amine concentration

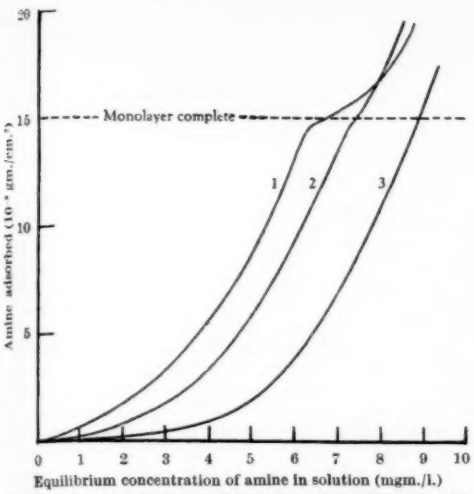

Fig. 2. Adsorption isotherms (30° C.) for *n*-dodecylamine in 10^{-3} *N* sodium perchlorate solution at *p*H 9·5 on (1) CuO, (2) NiO, (3) ZnO

ligand depends on the electronic structure of each. In particular, the ability to form π-bonds will significantly increase the bond-strength. Molecules containing an element such as sulphur in a low valency state can accommodate electrons from suitable levels in the metal ion in vacant levels and so give π-bonds. If the metal ions are in a solid lattice the electrons available for interacting with the ligand may either be isolated on the individual metal ions or they may be held in bands associated with the whole crystal if the solid is a semiconductor. In the latter case the relevant electron energy will be that at the surface and sensitive to lattice impurities in that region.

A large amount of data exists[4] showing that mercaptans, dialkyl sulphides, xanthates, dithiocarbamates and similar compounds containing divalent sulphur are adsorbed very strongly from solutions of suitable *p*H by solids containing metal ions which are in a suitable electronic state to form strong bonds with sulphur. With an oxidizing ion such as divalent copper, electron transfer often results, giving oxidized ligand and cuprous ions, whereas cuprous oxide, for example, simply adsorbs the reagent. If a solid has both anodic and cathodic areas on the surface, different adsorption behaviour would be expected at each kind of surface. In the case of galena and xanthate, adsorption occurs at the anodic regions[5] and greatly increased amounts are adsorbed when anodic bias is applied to the solid. The electron energy-levels are raised under anodic conditions so that the energy of electrons in the solid available for π-bond formation with vacant ligand orbitals is increased.

The views on the factors controlling chemisorption from solution set out above are strongly supported by experimental evidence. It is therefore possible to predict what reagents and treatment of the solid are likely to give strong adsorption under suitable conditions. This is of interest in infestigating the froth flotation process for separating minerals.

We thank the Department of Scientific and Industrial Research for a maintenance grant to one of us (G. H.) and the University of London Central Research Fund for a liquid scintillation counter.

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MODIFIED CELLULOSE ION EXCHANGERS AND THEIR USE IN RIBONUCLEIC ACID FRACTIONATION

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SINCE their introduction by Peterson and Sober in 1956[1] modified cellulose ion exchangers have been widely applied for the fractionation of polyelectrolytes like nucleic acids and proteins. Two types of resins particularly proved to be of value. The first one is an ion exchanger containing basic groups derived from triethanolamine bound to cellulose through reaction with epichlorohydrin. This resin, called Ecteola-cellulose, has frequently been used for nucleic acid fractionation[2–4].

A second type of cellulose adsorbent displayed particular merits for the chromatographic separation of proteins. It contains carboxymethyl (CM-cellulose), diethylaminoethyl (DEAE-cellulose), triethylaminoethyl (TEAE-cellulose) and phosphate (P-cellulose) groups. Among these DEAE-cellulose may also be used for the resolution of nucleic acid mixtures[5].

The obvious need for improvement of the available fractionation techniques in connexion with metabolic work on nucleic acids, urged us to explore the possibilities of cellulose ion exchangers more systematically.

In two earlier papers[6,7] we described a fractionation and purification of microsomal (*m*-RNA) and soluble ribonucleic acid (*s*-RNA) from rat liver on the resin Ecteola. Although the results were valuable for the establishment of certain metabolic interrelationships between the two kinds of nucleic acids, definite disadvantages of this resin soon became apparent. Following adsorption of a polymer of relatively high molecular weight (for example, microsomal RNA) on Ecteola columns, complete recovery cannot be achieved without application of eluants of high *p*H, degradative for RNA, and destroying the secondary structure of DNA. Consequently, the value of

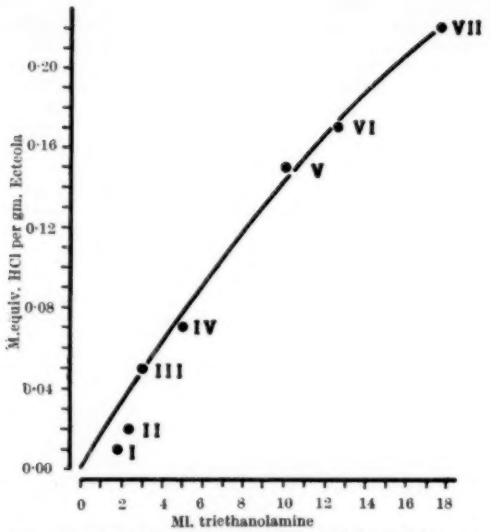

Fig. 1. Relationship between the amount of triethanolamine used for the preparation of Ecteola and the number of groups introduced as determined by potentiometric titration with hydrochloric acid. The resins were prepared as described in the text and designated Ecteola I to VII

Ecteola is rather limited although this resin is useful for the fractionation and isolation of smaller poly- and oligo-nucleotides and for analytical purposes. It is conceivable that a reduction in the density of binding sites on the exchanger would result in a weakening of the binding force between resin and the adsorbed polyanion. As a result the latter might then be eluted from the column by increasing the ionic strength of the eluant without necessarily raising the *p*H.

As an alternative such a weakening might be achieved by reducing the basicity of potential binding groups by introducing weaker bases than triethanolamine.

In this investigation both lines of approach have been followed. Various Ecteola resins with decreasing nitrogen content have been prepared and their capacities to fractionate soluble and microsomal RNA from rat liver have been compared. In addition, a new class of resins has been prepared by introducing nucleosides instead of triethanolamine into cellulose; the results of some preliminary fractionation experiments with the latter type of resins will be presented.

Experimental: Preparation of Ecteola-cellulose. Whatman cellulose powder (ashless, standard grade) was submitted for several hours to vigorous vibration in a sieve attached to the vibrator. Particles passing the sieve and having a diameter less than 0·05 mm. were used for the preparation of Ecteola according to Peterson and Sober[1].

Nucleoside cellulose was prepared by adding nucleoside instead of triethanolamine. Reactants were used in the following ratio: Whatman powder, 1 gm.; nucleoside, 1·9 m.mole; sodium hydroxide, 1 gm.; epichlorohydrin, 1 ml.; water, 2·5 ml.

Seven Ecteola resins were prepared according to Peterson and Sober[1], starting with constant amounts of cellulose (30 gm.) and epichlorohydrin (30 ml.) but increasing amounts of triethanolamine (1·75–17·5 ml.). Nitrogen content of the preparations as determined by potentiometric titration with hydrochloric acid, increased almost linearly with the amounts of triethanolamine, as shown in Fig. 1. Kjeldahl analysis yielded rather inaccurate results, probably due to the fact that very prolonged periods of heating with concentrated sulphuric acid were required for complete destruction. In most cases, however, about 90 per cent of all nitrogen groups determined by the Kjeldahl analysis could be accounted for by hydrochloric acid titration.

Fractionation of microsomal RNA from rat liver. Microsomal RNA was isolated from rat liver by means of phenol extraction[8,9], dialysed against 0·01 M phosphate, *p*H 5, and submitted to chromatography on the various Ecteola preparations. Some of the elution patterns, representative for our work on microsomal RNA, are illustrated in Fig. 2.

With increasing nitrogen content of the resins, a shift in these patterns towards the alkaline region is observed, and from Ecteola V onwards the amount of RNA requiring alkali for its elution rises. On Ecteola VII all the RNA emerges from the column in the alkaline region, indicating that fractionation experiments on resins of one particular nitrogen content only may not always yield a clear picture of the heterogeneity of the samples under investigation. Furthermore, it can be concluded from Fig. 2 that a fairly large proportion of *m*-RNA is recovered from various resins by sodium chloride elution. This fraction was designated as m_2-RNA, which means *m*-RNA recovered by washing with salt, although the varying proportions with which it is eluted in the salt region on various Ecteolas (Fig. 2) throws doubt on the homogeneity of this fraction. Ultracentrifugal analysis revealed that it is polydisperse[10].

Fractionation of soluble RNA. In earlier experiments[6] it was shown that a fraction of soluble RNA which is involved in the transfer of amino-acids to the microsomes, also appeared in the salt region when fractionated on Ecteola. The question could therefore be raised whether microsomal RNA contain

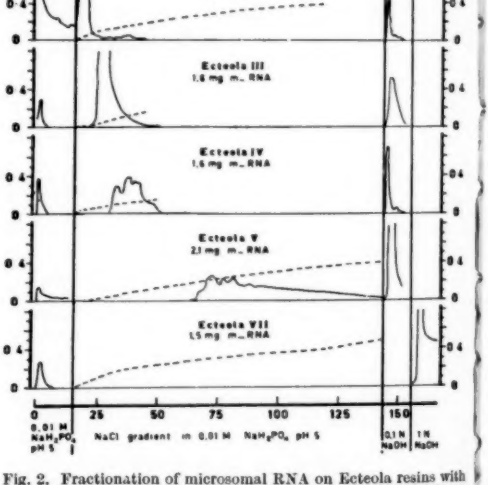

Fig. 2. Fractionation of microsomal RNA on Ecteola resins with increasing nitrogen content. Columns of 7 cm. length and 0·8 cm. diameter. A linear gradient elution system was established by applying a stock of 1 *M* NaCl–0·01 *M* phosphate (*p*H 5) solution and a mixing flask containing 1 l. of 0·01 *M* phosphate (*p*H 5). Optical density (O.D.) was read at 260 mμ

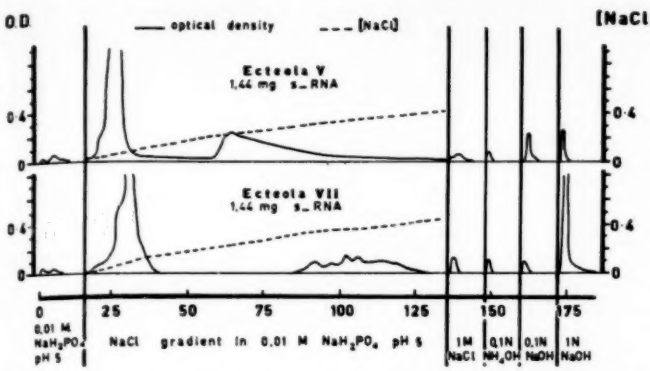

Fig. 3. Fractionation of soluble RNA on two Ecteola resins. For conditions, see legend, Fig. 2

soluble RNA, either as a native component or as a contamination due to inefficient washing of the microsomes. Support for the former assumption may be derived from communications by Smith *et al.*[11,12], who found that mouse-liver microsomes contain RNA (comprising 10–20 per cent of the total *m*-RNA) which will accept amino-acids, when incubated with enzymes at *p*H 5 under the usual conditions. Hence the behaviour of *s*-RNA on various Ecteola resins was examined, and some of the results are presented in Fig. 3. Chromatography of *s*-RNA on Ecteola V yields a fraction, earlier designated as s_3-RNA [6], which carries amino-acids and is indistinguishable from m_2-RNA, when fractionated similarly on Ecteola V.

A comparison of the elution patterns of *s*- and *m*-RNA on Ecteola VII, however, clearly demonstrates the non-identity of the fractions s_3 and m_2, and stresses once more the importance of using resins with varying densities of binding sites. A further characterization of the two fractions will be published elsewhere[10].

Two modifications in the elution procedure have been attempted: gradient elution with magnesium chloride in 0·01 *M* phosphate buffer of *p*H 5, and elution with increasing concentrations of phosphate, keeping the *p*H constant at 5. When *m*-RNA was fractionated on Ecteola IV only 10 per cent of the material could be recovered by applying the magnesium chloride gradient, the remaining 90 per cent appearing in the alkaline region. Similar results were obtained when the ionic strength was increased by applying a phosphate gradient.

Cellulose ion exchangers containing binding groups of lower basicity. The ease with which all kinds of groups may be introduced into cellulose suggested that nucleoside instead of triethanolamine might also react with cellulose through epichlorohydrin. According to this idea, four new ion exchangers have been prepared from adenosine, guanosine, cytidine and uridine, respectively. Preliminary results of *m*-RNA fractionation on the adenosine resin are illustrated in Fig. 4. More than 50 per cent of the polynucleotide adsorbed was recovered by applying a sodium chloride gradient elution system, the remainder by washing with ammonia and sodium hydroxide. *m*-RNA recovered by means of sodium chloride from the adenosine column and re-submitted to chromatography emerged from the column in exactly the same position, demonstrating a good reproducibility of this type of resin. Batches prepared at different times, however, did not always yield strictly identical fractionation patterns, necessitating a primary check before any new batch is going to be used.

Although the fractionation of *m*-RNA on guanosine cellulose has not been fully investigated so far, it appeared that *m*-RNA, when dissolved in 0·01 *M* phosphate, *p*H 5, is retained very weakly by the resin. Possibly elution has to be performed with buffers of very low ionic strength.

Uridine cellulose and particularly cytidine cellulose bind large proportions of *m*-RNA, recoverable by elution with alkali only.

Discussion. Ecteola and DEAE have a limited value for the fractionation of nucleic acids only due to the high pK' values of the two types of resins. (pK' of DEAE (in 0·5 *M* sodium chloride) is 9·5, that of Ecteola 7·5. Cellulose resins prepared from monoethanolamine (pK' 9) instead of triethanolamine (pK' 8·0) retained RNA more strongly than Ecteola did.) More favourable results might be expected by weakening the binding force between absorbent and polymer, for example, by replacing heteropolar linkages by van der Waals forces, hydrogen bonds, etc. In fact, a resin consisting of an insoluble polynucleotide seemed to meet these requirements ideally. Such a resin may be prepared by maximal loading of Ecteola with a synthetic polynucleotide of high molecular weight. Polyadenylic acid with a molecular weight of about 400,000, for example, is strongly bound by Ecteola[7], and can be recovered only by eluting with alkaline solutions. So far, exchangers of this type have not been investigated in this laboratory due to the fact that large amounts of polynucleotides are needed and were not available to us at the time. As an alternative, nucleosides were introduced into cellulose through reaction with epichlorohydrin, and preliminary results demonstrated that resins with satisfactory exchanging capacities were obtained. Of the four types of resins prepared, the purine cellulose exchangers showed the most promising fractionating properties. Uridine and cytidine cellulose retained

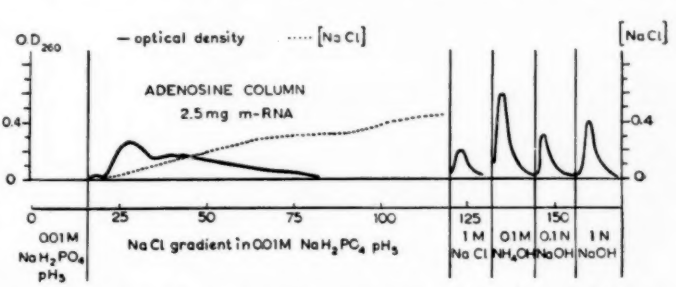

Fig. 4. Fractionation of microsomal RNA on adenosine cellulose. Column of 10 cm. length and 0·8 cm. diameter

m-RNA too strongly, and do not seem to have particular advantages over Ecteola.

The chromatographic behaviour of *m*-RNA on adenosine- and guanosine-cellulose is in accord with the lower dissociation constant of the NH_2-group of guanosine (pK'_a 1·6) as compared with that of adenosine (pK'_a 3·45) (ref. 13).

The extent to which ring opening of the purine and pyrimidine moieties occurs during preparation of the resin remains to be investigated. Strong alkali is required, and the temperature rises to more than 100° C. during the exothermic reaction between cellulose, epichlorohydrin and nucleoside. Potentiometric titration of adenosine cellulose did not reveal the presence of any additional basic groups and the pK'_a of the resin is the same as that of free adenosine. This may indicate that the purine structure remained unaltered during preparation of the ion exchanger. The strong retention of *m*-RNA by pyrimidine cellulose, however, throws doubt on this question so far as the pyrimidines are concerned.

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DIMER FORMATION AND THE TRANSFER OF GLYCEROL ACROSS THE ERYTHROCYTE MEMBRANE

By Dr. W. D. STEIN*

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A NUMBER of cell membranes have been shown to possess specialized systems which accelerate the movement down the concentration-gradient of specific metabolites. In the absence of such systems, these metabolites can cross the membrane slowly or not at all. Among such 'facilitated diffusion' systems[1] are those concerned in the movement of glucose into the human erythrocyte[2] and into muscle diaphragm[3] and the inward movement of sodium ions during the passage of the nerve impulse[4]. Most models that have been suggested for facilitated diffusion have involved hypothetical carriers in the cell membrane. The present investigation of the system which transfers glycerol across the human erythrocyte membrane[5] was initiated in the hope of isolating such a glycerol carrier. However, during the course of this work it has become clear that a model can be suggested which accounts for the facilitated diffusion of glycerol, without requiring the assumption of any membrane carrier at all.

It is perhaps useful to recall what constitutes the normal barrier to the movement of a molecule such as glycerol across the membrane, in the absence of any facilitated diffusion system[6]. The three hydroxyl groups of glycerol form strong hydrogen bonds with water. These hydrogen bonds anchor the glycerol in the aqueous phase, hindering its entrance into the hydrophobic lipid region of the membrane and hence reducing the rate at which it crosses the membrane. The membrane carriers that some previous workers have postulated have been 'alternative-hydrogen-bond formers' which can form hydrogen bonds with the glycerol molecules, competing with the water molecules. The carrier-glycerol complex has then its hydrogen-bonds internally saturated and can enter the cell membrane largely free of attached water molecules (Fig. 1*a*).

However, another method by which the glycerol molecule can have its hydrogen bond-forming capacity internally saturated is as follows (I am greatly indebted to Dr. D. A. Haydon of this Department for providing me with this idea, which is the core of the present theory): Two glycerol molecules may interact to form a dimer (Fig. 1*b*). Then each can act effectively as the 'carrier' for its fellow. On this basis I have derived the proposed model. I postulate the existence in the cell membrane of sites which bind pairs of glycerol molecules, thereby concentrating them and enabling them to interact with one another to form dimers. These dimers being free of attached water molecules (but still attached to the binding sites), are enabled to enter the hydrophobic region of the membrane. Inside the membrane the dimers dissociate from the binding site and can then traverse the membrane (either as dimers or, for the most part, having dissociated again into monomers). A summary of the evidence in favour of this model is presented here. Full details will be published elsewhere.

All the present measurements of red cell permeabilities have been made using the method of Orskov[7], in which the changes in optical density of a suspension of

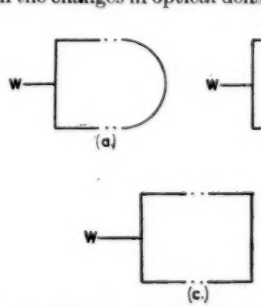

Fig. 1. Glycerol-'carrier' complexes: (*a*) glycerol + 'carrier'; (*b*) glycerol + glycerol; (*c*) glycerol + 1,3-dihydroxypropane. The left-hand molecule is, in each case, glycerol hydrogen-bonded to one water molecule

* Beit Memorial Research Fellow.

red blood cells on the addition of a solution of penetrating molecules is recorded. Analysis of the curves relating these changes in optical density with time shows that the curves are for the most part exponential functions. The half-time ($t_{\frac{1}{2}}$) of such a curve (the time taken for the galvanometer deflexion recording the optical density to fall to half its initial value) can be related to the reciprocal of the rate at which the permeant crosses the membrane[5].

Postulate 1. *The glycerol acceptor site is bivalent.* (*a*) The activity of the glycerol facilitator is a function of *p*H. In Fig. 2, the reciprocal of the half-time for the entry of glycerol into the cell is plotted against *p*H for a number of buffer systems. Curve *A* in Fig. 2 is drawn on the assumption that one proton combines with a univalent glycerol acceptor to form the inactive complex, for example:

$$F + H^+ \rightleftharpoons FH^+ \quad (1)$$

$$F + G \rightleftharpoons FG \quad (2)$$

where F is the facilitator in the form capable of accepting a glycerol molecule G, FH^+ is the inactive form, and FG is the glycerol-facilitator complex, which diffuses across the membrane. Curve *B* in Fig. 2 is drawn on the assumption that two protons are required to inactivate the glycerol-transferring system thus:

$$F + H^+ \rightleftharpoons FH^+ \quad (1)$$

$$FH^+ + H^+ \rightleftharpoons FH_2^{++} \quad (3)$$

$$FH_2^{++} \rightleftharpoons \text{inactive form} \quad (4)$$

$$F + G \rightleftharpoons FG \quad (2)$$

Clearly, it is this latter formulation that accords with the experimental results.

(*b*) Copper ions inhibit the glycerol facilitator. In Fig. 3, a plot of $1/t_{\frac{1}{2}}$ against *p*M is presented. The free metal ion concentration was maintained with lysine as the metal buffer, using the copper di-lysine/lysine equilibrium. (The methodology of such diligand buffers will be presented elsewhere.) Again curve *A* is drawn for the metal equilibrium analogous

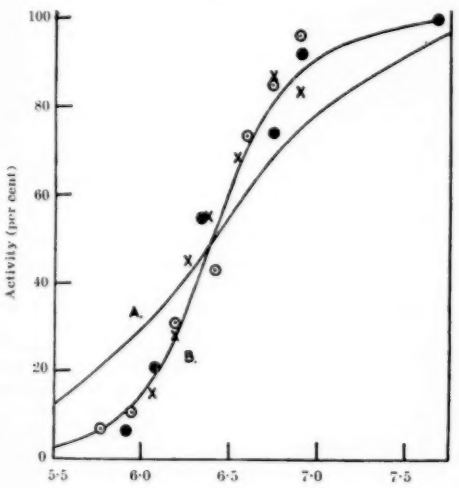

Fig. 2. Proton inhibition of the glycerol facilitator. Solid circles, histidine buffer; open circles, phosphate buffer; crosses, maleate buffer

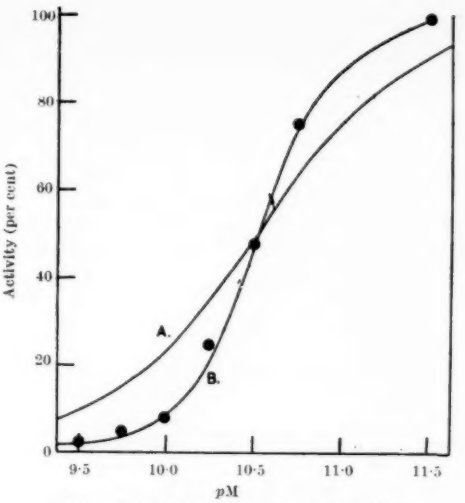

Fig. 3. Cu^{++} inhibition of the glycerol facilitator

to equations (1) and (2) involving a single copper ion, while curve *B* is drawn on the assumption that two copper ions are required to produce inhibition. Again the bivalent nature of the facilitator is manifest.

(*c*) Both these experimental procedures are mute, however, as to the number of glycerol molecules present in the glycerol-facilitator complex. To investigate this, the activity of the system as a function of glycerol concentration was investigated. Red cells were equilibrated in successively increasing concentrations of glycerol and, after equilibration, a further quantity of glycerol, the same in each experiment, was injected into the suspension, and the optical density changes recorded. A theoretical analysis of the changes of cell volume with time in these conditions gave the following results:

The penetration of diethylene glycol, a molecule for which there is no reason to suspect that a facilitated diffusion system exists, is, at low concentrations, in very fair accord with the theory for penetration by simple diffusion. Somewhat surprisingly, the results for the penetration of glycerol also accord with simple diffusion theory. If, on the other hand, the theory for penetration by binding with a univalent carrier (equation (2)) is applied to the data for glycerol penetration, the absurd result is obtained that the dissociation constant of the carrier-glycerol complex is large and negative, that is, there is no affinity between glycerol and the membrane carrier. If, however, the bivalent theory is applied according to the equilibrium:

$$F + G \rightleftharpoons FG \quad (2)$$

$$FG + G \rightleftharpoons FG_2 \quad (5)$$

where FG_2 is the penetrating species, good accord is obtained below 1·3 *M* glycerol and an affinity constant of reasonable magnitude obtained.

The affinity constant for the first stage in the formation of the bivalent complex, that is, the interaction of single glycerol molecules with the acceptor sites according to equation (2) can be derived and is found to be 1·0 *M*. (The significance of this value will be discussed under postulate 3).

Thus it would appear that glycerol penetrates either by simple diffusion or by interaction with a bivalent

acceptor, the results excluding the univalent acceptor model. But penetration by simple diffusion can also be excluded since glycerol penetration, but not that of diethylene glycol, is inhibited by protons (Fig. 2), by metal ions (Fig. 3), by substrate analogues (Fig. 4) and by a variety of other agents[8]. (See also an earlier paper[5] using a somewhat different experimental approach, where saturation of a membrane acceptor by glycerol could be demonstrated.) Thus the kinetic data demand that the facilitated diffusion system is bivalent towards the substrate glycerol as well as towards the inhibitors H^+ and Cu^{++}.

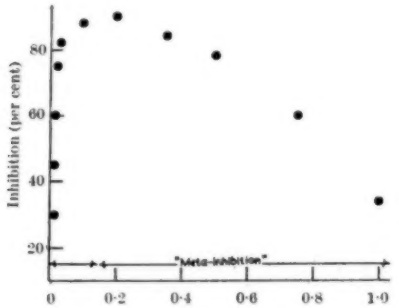

Fig. 4. Inhibition and meta-inhibition by 1,3-dihydroxypropane. Glycerol concentration fixed at 0·1 *M*, glycol concentration 0–1 *M* (abscissa)

Postulate 2. *Dimer formation leads to an increased rate of penetration.* The glycol 1,3-dihydroxypropane (or 103) is a competitive inhibitor of the glycerol facilitator. At a concentration of 0·01 *M* 103 (equal to one-tenth that of the glycerol added), the rate of entry of glycerol is inhibited by 50 per cent. But as the concentration of 103 is increased, the degree of inhibition passes through a maximum and then decreases again until at 1 *M* 103 the system is only 20 per cent inhibited (Fig. 4). This falling-off in the effectiveness of an inhibitor at high concentrations of inhibitor, or 'meta-inhibition', has here proved difficult to interpret, until now. However, let us suppose that glycerol and the glycol in the bulk of the solution interact as in Fig. 1c to form a dimer, albeit to a very slight extent. Then this complex will have a greatly increased rate of penetration across the membrane, as it has only one free hydrogen-bonding site available for binding to the water. On this view, then, meta-inhibition should bear no relation to the facilitated diffusion system. In fact, at concentrations of copper ions sufficient to block facilitated glycerol transfer completely, addition of 103 appears to restore the rapid transfer rate, and the same can be shown for hydrogen-ion inhibited cell preparations. Even more striking are the experiments using bovine blood. The bovine erythrocyte lacks the facilitated diffusion system for glycerol. However, if to a suspension of bovine red cells is added 0·5 *M* 103, there is a dramatic increase in the rate of transfer of glycerol and at a level of 1 *M* 103 beef red cells are, so far as their glycerol permeability is concerned, indistinguishable from normal human red cells (Fig. 5). 103 can, therefore, act as a membrane carrier for glycerol in both bovine cells and in inhibited human red cells.

Postulate 3. *The properties of the glycerol-binding site require no improbable assumptions as to energetics.*

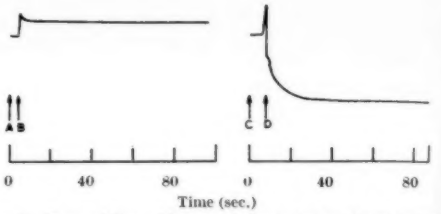

Fig. 5. Permeability of bovine erythrocytes to glycerol in the absence and presence of 1,3-dihydroxypropane. These are tracings of the experimental records of the optical density changes. At *A*, a suspension of bovine erythrocytes is added; and at *B*, glycerol injected to give a final concentration of 0·1 *M*. At *C*, the solution is changed and replaced by bovine erythrocytes equilibrated in 1 *M* 1,3-dihydroxypropane. At *D*, glycerol to 0·1 *M* is injected into this suspension

Direct experimental studies of glycerol dimer formation in dilute aqueous solutions are lacking. However, an estimate of the probable extent of dimer formation can be reached by considering the competition between water molecules and glycol molecules for each other according to the equilibrium:

$$AW + BW \rightleftharpoons AB + W_2 \qquad (6)$$

where *A* and *B* are hydrogen-bonding molecules interacting with water *W* to form *AW* and *BW*, and with each other to form *AB*. W_2 is the water dimer. Assume that all the hydrogen bonds *AW*, *BW*, *AB* and *WW* are equivalent and that only hydrogen bonds are concerned in the equilibrium. We can then apply the law of mass action to the equilibrium (6), and show that for a glycol concentration of 1 *M* and glycerol at 0·1 *M*, the extent of dimer formation (of the type of Fig. 1c) is some 0·1 per cent of the glycerol molecules. Now, a monohydroxy compound, such as in 1c, would be expected to penetrate the membrane about 10^4 times as fast as glycerol (ref. 6). Thus the increase in glycerol permeability in 1 *M* glycol should be then about $10^4 \times 10^{-3} = 10$-fold. In fact, at 1 *M* 103, glycerol penetration into bovine erythrocytes is speeded up some fifty-fold. This discrepancy between these two figures is not excessive considering the very drastic assumptions made in applying the law of mass action to equation (6). (In particular, the contribution to dimer formation by —CH_2 . . . CH_2— interaction has been ignored.) By similar arguments, it can be shown that to account for the experimentally observed value (1·0 *M* at 20° C.) of the affinity constant of single glycerol molecules for the binding site, 1,500 cal./mole of free energy must arise from binding other than at the hydroxyl group. This is a satisfactory figure for the interaction of 2 or 3 of the CH— residues of glycerol with hydrophobic sites on the glycerol acceptor.

We have seen then that interaction between glycerol and 1,2-dihydroxypropane, a molecule which can form dimers with glycerol, leads to a great increase in the rate of penetration of glycerol across the bovine erythrocyte membrane and that the extent of this increase is consistent with the extent of dimer formation to be expected in such solutions. Also, during the transfer of glycerol by the facilitated diffusion system across the human erythrocyte membrane, two glycerol molecules are bound at each acceptor site to form the penetrating complex. Finally, estimates of the energy of such interactions show that the degree of concentration of the glycerol molecules required for the system to operate is not beyond the capacity of a simple enzyme-like binding site.

The results strongly suggest, therefore, that the facilitated diffusion of glycerol occurs as a result of dimer formation between pairs of glycerol molecules bound at acceptor sites in the erythrocyte membrane.

It will be of interest to investigate whether this model can be applied more generally so as to include other facilitated diffusion systems.

I should like to thank Prof. J. F. Danielli (of King's College, London, where this work was commenced), and Prof. F. J. W. Roughton (of the Department of Colloid Science, Cambridge), for their hospitality in providing laboratory facilities for this work. Dr. D. A. Haydon and Dr. J. C. Kernohan of this Department were of invaluable help with their stimulating comments and discussion. I am indebted to the Royal Society and to Imperial Chemical Industries, Ltd., for financial assistance towards the cost of chemicals and apparatus.

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SOLUBILITY OF DENATURED HÆMOGLOBIN VARIANTS IN ACID BUFFERS

By I. D. FRASER and Dr. A. B. RAPER
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WHEN methanol-fixed smears of human cord blood are exposed for 90 sec. at 37° C. to a 0·17 *M* phosphate–citrate buffer, washed, and afterwards stained with a Romanowsky dye, the degree of eosinophilic staining of individual erythrocytes varies from a negligible amount ('ghosts') to full coloration[1]. Erythrocytes in the blood of adults all appear as 'ghosts', and it is assumed that the fully coloured cells in cord blood are of fœtal type. It has not been proved that the retention of staining properties is in fact an expression of the insolubility of fœtal hæmoglobin (*F*) in the acid buffer, but we have obtained indirect evidence that this is so, and that the process allows a visualization of the hæmoglobin *F* present in individual cells.

The differential staining effect can only be obtained after denaturation of the hæmoglobin, either by methanol, acetone or heat. We therefore tested the solubility of hæmoglobins *A* and *F*, after acetone denaturation, in two ways: (*a*) after partial separation of a 1 : 1 mixture of hæmoglobins *A* and *F* by electrophoresis on 3 *MM* Whatman filter paper at *p*H 8·6, followed by immersion of the strip in acetone; (*b*) after precipitating a hæmolysate of cord blood with acetone. In the former case, immersion of the strip in the acid buffer for 2 hr. caused elution of most of the more anodically situated hæmoglobin (largely *A*), leaving the remainder (mostly hæmoglobin *F*) undissolved. In the second case, complete solution of 10 mgm. of denatured hæmoglobin *A* could be obtained in 10 ml. of the acid buffer, while with 10 mgm. of denatured hæmoglobin obtained from a cord blood hæmolysate containing 32 per cent of hæmoglobin *A*, only 28 per cent of the hæmoglobin was soluble in 10 ml. of acid buffer. Details of these experiments will be given later, but their results allowed us to proceed to investigate the distribution of hæmoglobin *F* in the erythrocytes of subjects with thalassæmia.

In smears from subjects with thalassæmia major, all the erythrocytes retained their hæmoglobin staining properties, reacting exactly as fœtal cells do (Fig. 1), when due allowance is made for the reduced thickness of many of the cells. In thalassæmia minor subjects in whom there was a measurable amount of hæmoglobin *F*, there was partial retention of hæmoglobin staining, and the degree of this did not vary from cell to cell (Fig. 2). This homogeneous picture was quite different from that given by cord blood or the blood of neonates. We interpret this as indicating that beyond the age of infancy hæmoglobins *F*

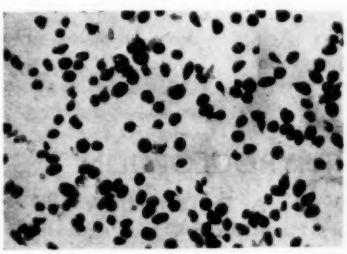


Fig. 1

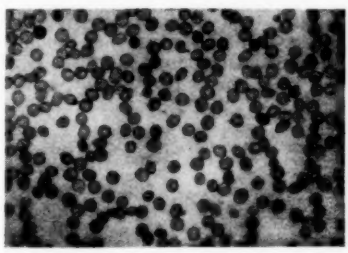
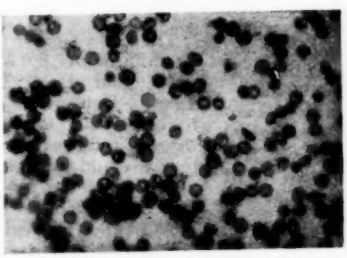


Fig. 2



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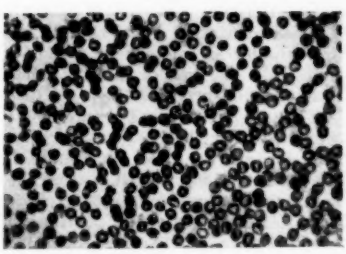

Fig. 4

Methanol-fixed blood smears, exposed to phosphate–citrate buffer at *p*H 3·4, and afterwards stained by May–Grünwald stain. (1) Thalassæmia major. (2) Thalassæmia minor. (3) Hæmoglobin *S*/*C* disease. (4) Sickle-cell trait

and *A* must be synthesized together in each erythrocyte precursor. This double synthesis must occur in the marrow, the only erythropoietic organ in subjects with thalassæmia minor; and although there may be some extramedullary erythropoiesis in the case of thalassæmia major, exactly the same double synthesis must occur in other sites as in the marrow, since only one sort of cell appears in the blood.

It must be mentioned, however, that the proportion of 'insoluble' hæmoglobin retained in the erythrocytes from subjects of thalassæmia minor appeared excessive in relation to the low hæmoglobin *F* content of hæmolysates from these subjects. It is possible, therefore, that a part of their hæmoglobin *A*, though reacting normally to electrophoresis and alkali denaturation, may in fact be an aberrant molecular species, as postulated by Ingram and Stretton[2]. Some support for this is provided by the findings in congenital spherocytosis, where with proportions of hæmoglobin *F* in the same low range as in thalassæmia minor, there was no retention of hæmoglobin in the erythrocytes.

It was of interest to discover whether other hæmoglobin variants could be visualized in the same way as hæmoglobin *F*. Blood smears from subjects with various hæmoglobinopathies were submitted to the acid-lysis test, using buffers of several *p*H values. The results are shown in Table 1.

Where hæmoglobins *F* and *A* occur together, there was retention of hæmoglobin in the cells, and the degree of retention was roughly proportional to the amount of hæmoglobin *F*, with the exception, already noticed, of thalassæmia minor. The retention of hæmoglobin in the erythrocytes of a subject displaying hæmoglobin *H* is complicated by the facts that some hæmoglobin *F* was present, and that such persons are heterozygous for the thalassæmia gene. Hæmoglobins *C* and *D* are indistinguishable in their reaction from hæmoglobin *A*, while hæmoglobin *E* appears to be less soluble than hæmoglobin *A*. In the case of a subject with hæmoglobin *S/C* disease, every cell appeared to have retained about half its complement of hæmoglobin (Fig. 3). Since hæmoglobin *C* has been shown to be eluted from the cells of a hæmoglobin *C* trait carrier (*AC*), the insoluble fraction was presumed to be hæmoglobin *S*. Yet when the test was applied to sickle-cell trait (*AS*) subjects, carrying less hæmoglobin *S* than occurs in hæmoglobin *S/C* disease, almost the whole of the hæmoglobin appeared to be retained in every cell at *p*H 3·4 (Fig. 4) though all was eluted at *p*H 3·0. In sickle-cell anæmia the retention was even greater, being evident at *p*H 3·0. Some part of the retention in the latter case might be ascribed to the small amount of hæmoglobin *F* present, but this will not suffice to explain the difference between the *A* + *S* and the *S* + *C* subjects. We assume that either in its native state, or more probably during methanol denaturation, hæmoglobin *S* forms an insoluble complex with hæmoglobin *A* but not with hæmoglobin *C*.

Table 1. Retention of Hæmoglobin Variants in Fixed Erythrocytes exposed to Acid Buffers

Cells containing hæmoglobins	Proportion of hæmoglobin *F* (per cent)	Retention of hæmoglobin in erythrocytes treated at *p*H: 3·4	3·0	2·0
A	< 1	0	0	0
F + *A* (16-week fœtus)	94	+ + +	+ +	+
F + *A* (thalassæmia major)	70	+ +	+	0
A + *F* (thalassæmia minor)	2·7–3·5	+	...	...
A + *F* (congenital spherocytosis)	3·3–3·5	0	0	0
S + *F* (sickle-cell anæmia)	8–17	+ +	+ +	0
A + *S* (30–45 per cent) (sickle-cell trait)	< 1	+ +	0	0
C + *S* (50 per cent)	...	+	0	0
A + *C* (40 per cent)	< 1	0	0	0
A + *D* (45 per cent)	< 1	0	0	0
A + *E* (30 per cent)	< 1	±	0	0
A + *H* (15 per cent)	3	+ +	0	0

+ + +, complete retention; + +, almost complete retention; +, about 50 per cent retention; ±, less then 33 per cent retention; 0, no retention.

We acknowledge receipt of specimens generously sent by Drs. E. R. Huehns, D. Stansfield, J. A. M. Ager, D. Speed and H. Lehmann.

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THYROGLOBULIN

By Dr. A. D. LITONJUA*
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Effect of Acid

EDELHOCH has shown that alkali produces new sedimentable species of thyroglobulin which are smaller in molecular weight than the original material[1]. It was of interest, therefore, to find out if acid media produce the same dissociation effect.

Thyroglobulin was prepared in 1 per cent concentration in three buffer solutions according to a method in ref. 2: (1) 0·1 *M* phosphate buffer at *p*H 8·0; (2) 0·25 *M* acetate buffer at *p*H 3·5; and (3) 0·1 *M* hydrochloric acid–potassium chloride buffer at *p*H 1·5. Aliquots of each of these preparations were examined in the Spinco model *E* ultracentrifuge. Viscosity measurements were made in an Ostwald viscometer using standard procedures and calculations for intrinsic viscosities (ηi), expressed[2a] in centipoises per gm. thyroglobulin ($\eta i/c.$). Experimental temperature was 25° C.

The difference in the intrinsic viscosities of thyroglobulin at *p*H 8·0 and *p*H 3·5 was less than that at *p*H 8·0 and *p*H 1·5 (Fig. 1). Precipitation work to be described elsewhere showed the *p*H 3·5 preparation to be 50–60 per cent denatured, whereas *p*H 1·5 thyroglobulin was completely denatured. The latter result could account for the great difference in viscosity of the *p*H 1·5 material from either of the two preparations.

At *p*H 8·0, thyroglobulin showed three components, whereas two boundaries were evident at lower *p*H (Fig. 2). Molecular weight calculations using sedimentation values and viscosities of the different thyroglobulin preparations were made[2b]. The partial specific volume for thyroglobulin was taken as

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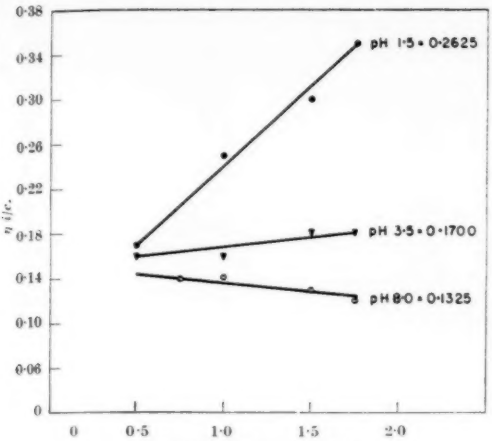
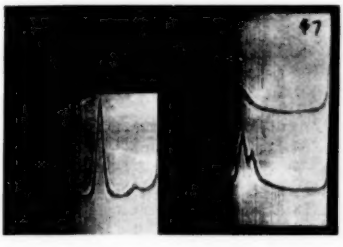


Fig. 1



(*A*) (*B*)

Fig. 2. Ultracentrifugal patterns of thyroglobulin. Direction of migration from left. All proteins, 1 per cent concentration. (*A*) Duration of spin, about 90 min. Thyroglobulin in 0·1 *M* phosphate buffer, *p*H 8·0. (*B*) Duration of spin, 47 min. Upper, thyroglobulin in 0·25 *M* acetate buffer, *p*H 3·5; lower, thyroglobulin in 0·1 *M* hydrochloric acid–potassium chloride buffer, *p*H 1·5

0·714 (ref. 1). Table 1 summarizes the different results obtained, together with the relative concentrations of each component derived from the areas under the peaks. At *p*H 3·5, a species, which was approximately one-half the molecular weight of the $S_{20,w}$ = 14·7 component of *p*H 8·0 thyroglobulin, was formed. The same finding was apparent at *p*H 1·5. The relative concentrations of the sedimentable components at *p*H 3·5 and *p*H 1·5 are approximately the same, so the $S_{20,w}$ = 8·0 or 8·3 components could not be denatured forms of thyroglobulin. It could be more plausible to explain these forms as arising from a dissociation phenomenon analogous to that observed by Edelhoch[1].

Table 1

	$S_{20,w}$	Relative concentration (per cent)	Molecular weight
Thyroglobulin, at:			
(1) *p*H 8·0	3·7	6	
	14·7	86	690,000
	22·2	8	
(2) *p*H 3·5	8·3	76	317,000
	11·3	24	
(3) *p*H 1·5	8·0	65	336,000
	11·6	35	

Evidence of Thyroid Protease Action

A MATERIAL rich in proteolytic activity against hæmoglobin can be obtained from thyroid glands by ammonium sulphate precipitation followed by methanol–sodium chloride fractionation[4]. However, this preparation is relatively ineffective against thyroglobulin[5]. Although an alternative method of thyroid protease preparation by acetone fractionation[7] provides substantial improvements in the yield and purity of the proteolytic activity against hæmoglobin, the activity against thyroglobulin is not affected, nor does treatment of the substrate by urea improve this activity. In previous work, the methods used for following proteolysis were those aimed at the detection of small peptide fragments or the known iodinated thyroid hormones. If, however, the main action of thyroid protease is to hydrolyse only a few crucial bonds in thyroglobulin, thus enabling other enzymes to act, then it is rather easy to see why these methods depict the enzyme as relatively ineffective against thyroglobulin. This report provides evidence for the premise.

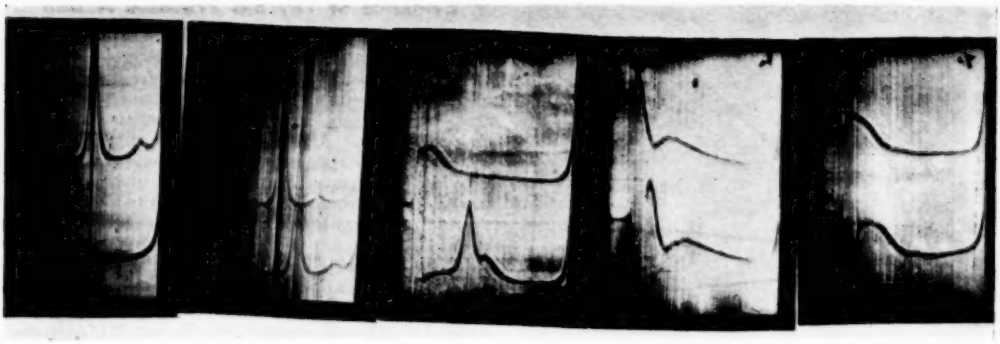
Sheep thyroglobulin, prepared according to the method in ref. 2, was dissolved in 1 per cent concentration in either 0·1 *M* phosphate buffer at *p*H 7·0 or in 0·25 *M* acetate buffer at *p*H 3·5. The upper curve in Fig. 3*A* and the lower curve in Fig. 3*C* show the ultracentrifugal patterns of each thyroglobulin preparation. Thyroid protease showed one peak in either buffer (Fig. 3*A*, lower and Fig. 3*C*, upper).

After 2- and 6-hr. incubation at 37° C. in the Dubnoff shaker, aliquots of the *p*H 3·5 thyroglobulin and *p*H 7 thyroglobulin, each incubated with thyroid protease, were removed for examination in the Spinco model *E* ultracentrifuge and for viscosity investigation using standard procedures[3a]. Fig. 3*B* shows no change in the ultracentrifugal patterns of the *p*H 7·0 thyroglobulin hydrolysate. However, marked alterations were seen in *p*H 3·5 thyroglobulin (Fig. 3, *D* and *E*).

Molecular weights can be determined from the sedimentation constants and viscosities[3b]. The

Table 2

	$S_{20,w}$	Composition (relative percentage)	Intrinsic viscosity (centipoises)	Approximate molecular weight
I. At *p*H 8·0				
1. Thyroglobulin, control	3·7	6	0·1299	
	14·7	86		690,000
	22·2	9		
2. Thyroid protease control	3·02	—	—	—
3. 2-hr. hydrolysate	2·9 (protease?)	36	0·1778	
	13·83	> 56		680,000
	20·70	< 18		
4. 6-hr. hydrolysate	3·02 (protease?)	32	0·1841	
	13·83	> 61		720,000
	20·80	< 7		
II. At *p*H 3·5				
1. Thyroglobulin, control	8·3	76	0·1626	317,000
	11·3	24		
2. Thyroid protease, control	2·51	—	—	
3. 2-hr. hydrolysate	1·96	> 52	0·1152	29,000
	17·44	< 48		
4. 6-hr. hydrolysate	1·42	> 52	0·0954	1,500
	15·48	< 48		



(A) (B) (C) (D) (E)

Fig. 3. Ultracentrifuge patterns. Centrifugation at 56,200 r.p.m. Direction of migration from left. (*A*) Duration of spin, 104 min. Upper, thyroglobulin in 0·1 *M* phosphate buffer, *p*H 8·0; lower, thyroid protease in same buffer. (*B*) Duration of spin, 90 min. Upper, 2 hr. thyroglobulin hydrolysate in 0·1 *M* phosphate buffer, *p*H 8·0; lower, 6-hr. hydrolysate, same buffer. (*C*) Duration of spin, 105 min. Upper, thyroid protease in 0·25 *M* acetate buffer, *p*H 3·5; lower, thyroglobulin in same buffer. (*D*) Duration of spin, 27 min. Upper, 2-hr. thyroglobulin hydrolysate in 0·25 *M* acetate buffer, *p*H 3·5.; lower, 6-hr. hydrolysate. (*E*) Duration of spin, 95 min. Upper, 2-hr. hydrolysate of *p*H 3·5 material; lower, 6-hr. hydrolysate. Faster moving component not visible in field at this point

viscosity measurements in this work were made on heterogeneous preparations, hence the calculated viscosity was the sum of viscosities of each component in the preparation. It was felt that this method, while not precisely accurate for molecular weight determinations, could give an approximation of what happened to the major component of the thyroglobulin preparation. As Table 2 shows, when no action of the enzyme was evident, the calculated molecular weight of the main thyroglobulin peak remained within the value of the control. The value of 0·714 was used as the partial specific volume of thyroglobulin[1]. Table 2 shows more than 50 per cent of *p*H 3·5 thyroglobulin reduced in a 2-hr. incubation to a component having a molecular weight less than one-tenth of the control. A further degradation occurred with progression of the incubation period.

With these observations and other work, it is postulated that thyroid protease exerts a 'priming' action on thyroglobulin by breaking it down into small polypeptide fragments. The formation afterwards of the thyroid hormones could take place as the result of such processes as the action of thyroid peptidases, action of serum peptidases, or reversal of biosynthetic pathways.

I wish to express my gratitude to Drs. M. J. Spiro and J. B. Stanbury for their advice and helpful suggestions.

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CORRELATION BETWEEN GROWTH-RATE OF THE SUCKLING OF VARIOUS SPECIES AND THE PERCENTAGE OF TOTAL CALORIES FROM PROTEIN IN THE MILK

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BUNGE[1] proposed that there is a direct correlation between the growth-rate of the sucklings of several species (expressed as the number of days required for doubling the birth-weight) and the percentages of protein and ash in the fluid milk.

As a normal animal ingests only the amount of food necessary to satisfy the caloric requirement, maximal growth will occur only if this intake contains an adequate amount of protein (and all other nutritionally essential materials as well) for metabolic needs and for formation of new tissue. Powers[2] pointed out that as the requirement for calories determines intake of food, the percentage of total calories derived from protein is a better measure of the protein content of milk.

Using the percentage of total calories from protein as the criterion of protein content of milk, Powers[2] concluded that there is no correlation between the rate of growth of the suckling and the protein content. Brodie[4] concluded "that the situation is too complex for simple generalization although speed of growth, composition and physiological age are important factors in determining the evolutionary trend of milk composition".

Available data indicate that there is an inverse linear correlation between the percentage of total

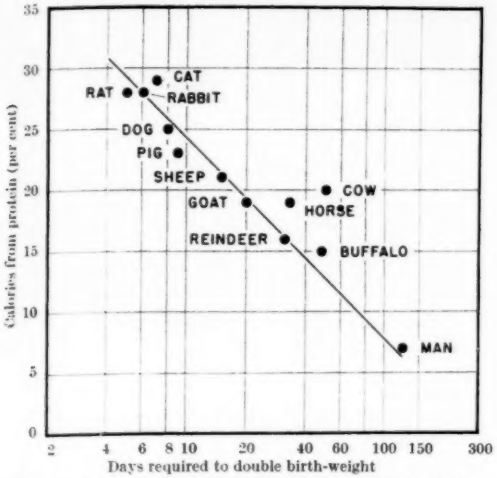

Fig. 1. The correlation between the percentage of total calories from protein and the number of days required to double the birth-weight. The formula of the line fitted by method of least squares is $y = 41 - 16{\cdot}5 \log x$ (data for cow not included)

calories supplied by protein in the milk and the logarithm of the number of days required to double the birth-weight of several species. The results for all species for which presumably reliable estimates of both variables have been found are listed in Table 1. A plot based on these results is shown in Fig. 1.

Early growth tends to follow the first-order reaction-rate[4]:

$$\frac{dW}{dt} = KW$$

where W is weight; t is time; K is first-order reaction-constant from which the following expression may be derived:

$$K = \frac{0{\cdot}693}{t_2}$$

where t_2 is days required to double the birth-weight.

The correlation between the log of K versus percentage calories from protein is positive and linear.

The caloric contents were calculated, using the factors 9, 4 and 4 cal./gm. for fat, protein and carbohydrate. Values for the time required for doubling the birth-weight were selected from work in which, so far as possible, optimal conditions were maintained. The values listed are considered to be the average for different varieties of a species.

The results indicate that the same relationship between protein supplied by the milk and rate of growth of various species is also true in man. The cow, however, appears to be an exception. In this species the percentage calories supplied by the protein of the milk is considerably greater than would be expected from the growth-rate. An excess of milk protein in relation to growth-rate is present, to a lesser extent, in the horse and buffalo. These three animals are herbivores with relatively slow growth-rates. A possible explanation for the excess of protein in their milks is that the food of the sucklings is also forage, and the higher protein content of the milk compensates for the lower protein content of the forage.

The elephant, not listed in Table 1, is of particular interest. Elephant milk has about the same protein content as human milk (7 per cent of calories)[3], but no exact information on rate of growth could be found. The Indian elephant is weaned at 4–5 years, puberty occurs at 14–15 years in the male and first calving at 15–16 years; full maturity is reached at the age of 40 and the life-span ranges up to 75 years. These similarities to man indicate that the elephant and human beings may have, during early life, similar rates of growth, predictable from the similar low protein content of the milk of these species.

Table 1. Percentage of Calories from Protein in the Milk and Growth-Rate (expressed as Days required to double Birth-weight) of Various Species

Species	Total calories from protein (per cent)	Refs.	Days to double birth-weight	Refs.
Man	7	11	125	6, 7
Buffalo	15	12	48	17
Reindeer	16	12	31	3
Horse	19	12	33	18
Goat	19	11	20	12, 3
Cow	20	11	50	28
Sheep	21	12	15	19, 3
Pig	23	13	9	20
Dog	25	14	8	28, 2
Rabbit	28	15	6	21
Rat	28	16	5	8
Cat	29	15, 2	7	22, 4

According to Brodie[4] the speed of growth is an important factor in determining the evolutionary trend of milk composition rather than milk composition having a role in determining the growth-rate. That the protein content of milk is not the factor limiting the rate of growth was pointed out by Minot[5], as increasing the protein intake above that supplied by the milk has not been shown to cause an increase in the rate of growth. The only results have been obtained from human studies. The growth-curves of breast-fed infants during the period required to double the birth-weight[6] were practically identical to those obtained in an earlier investigation at the same location with a large number of infants preponderantly fed cow's milk formulæ supplying 2–3 times as much protein[7].

A rough approximation of the amount of protein supplied by milk in relation to needs of the suckling may be made by comparing the protein intake from milk with the protein requirement after weaning. The results in Table 2 indicate that the protein requirement of the weanlings of various species for optimal growth is about half the amount of protein supplied by the milk. A larger amount of protein supplied by milk, as compared with the dietary requirement after weaning, is necessary for the rapid rate of growth after birth in comparison with the markedly slower growth-rate after weaning[4]. In man, requirement for protein per kgm. body-weight after birth is 1·9 times the requirement at 6 months[9].

Table 2. Comparison of the Percentage of Calories from Protein in Milks with Requirement for Optimal Growth after Weaning

Species	Calories from protein (per cent)		Ratio	Source of protein	Refs.
	In milk (1)	Post-weaning requirement (2)			
Man	7·2	4·4	0·61	Cow's milk	9
Pig	23	12	0·52	Cow's milk	23
Dog	25	17	0·68	Corn, yeast, casein	24
Rat	28	15	0·54	Casein plus 0·5 per cent methionine	25
Cat	29	19	0·66	Casein	26

These results indicate that generally the milks of the various species supply an amount of protein adequate for maximal growth but not much more.

The advantage of the evolutionary development of a milk composition which supplies enough protein for maximal growth of the offspring is evident. Nature's conservatism in limiting protein to the suckling is undoubtedly of benefit to the mother. The strain of lactation would be increased if excess protein were excreted in the milk, in view of the mother's limited stores of body protein and possible difficulties in obtaining an adequate protein intake to maintain positive nitrogen balance.

It is also possible that an adequate, though limited, protein intake may be of benefit to the offspring as well. Holt[10] has reviewed the recent literature on the effects of various protein-levels in the diet on body composition, enzyme-levels, effects of stresses, diseases and starvation and incidence of tumours. In several of these investigations diets supplying low or normal protein intake were found to be advantageous in comparison with those with higher amounts.

At the present time there is disagreement about the most advantageous level of protein intake of artificially fed infants. Gyorgy[27], a proponent of lower protein intakes for artificially fed infants, has used teleology as an argument; that is, there is a nutritional advantage associated with the low protein content of human milk because of purpose in Nature.

This teleological argument is strengthened by the present work, which supports the point of view that the same relationship between protein intake and growth-rate found in most other species is also true in man.

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TREATMENT OF HOPS (*HUMULUS LUPULUS*, L.) WITH GIBBERELLIC ACID

Effect of Spraying with Gibberellic Acid on Yield and Composition

NASH AND MULLANEY[1] have described trials in which hops were treated with various concentrations of gibberellic acid. The maximum effect was observed when the plants were sprayed at the time of burr formation and accordingly a large-scale trial was carried out in which the Australian commercial variety Pride of Ringwood was sprayed with 12·5 p.p.m. of the potassium salt of gibberellic acid at this time. At harvest, the yield of the treated hops was 39 per cent higher than that of the controls, but on analysis the treated hops contained only 1·8 per cent of α-acid on which the brewing value largely depends, as compared with 10·2 per cent of α-acids in the controls. The overall yield of useful material for brewing was therefore depressed considerably by the treatment. It appeared possible, however, that the treated hops might have appeared ripe prematurely and that if they had been left in the field longer the resin content might have risen to the same level as that found in the controls.

The results reported here are of a similar field trial conducted under English conditions, using the variety Bullion (*Q*43). Samples were taken throughout the ripening period until resin synthesis was demonstrably complete. Twenty-five hills of two-year-old plants of the variety Bullion grown in the Withersdane Garden, Wye College, were sprayed with a solution containing 12·5 p.p.m. of the potassium salt of gibberellic acid on July 19, 1960, at which time the majority of the stigmas of the female plants were about $\frac{1}{8}$ in. long. Twenty-five similar hills served as the controls. Within a week of application the treated plants showed differences from the controls in their growth and development. The new leaves were noticeably paler green and the laterals were longer with more extended internodes. Furthermore, the cones in the treated plants developed more rapidly than those of the untreated plants, with the result that, 15 days after spraying, the dry weight per 100 cones of the treated hops was 4·32 gm. compared with 1·40 gm. with the controls. A summary of the analytical data obtained with these and later samples are given in Table 1. With the immature samples the normal methods of analysis for α-acid were insufficiently sensitive, but when the resin obtained from these hops was examined by ion-exchange chromatography[2] the presence of α-acids in the treated hops and their absence in the controls was clearly demonstrated. At the time of the second sampling (August 17, 1960), the visual difference between the treated and control samples had largely disappeared, but the treated hops still contained more resin and α-acids than the controls. Both samples contained a trace of steam-volatile oil, though when this was examined by gas chromatography none of the com-

ponents of the essential oil of mature hops could be detected. At the third sampling (August 31, 1960), resin synthesis in both treated and control samples appeared to be complete. On the other hand, the essential oils continued to be synthesized until harvest (September 29, 1960), a point which has not emerged from earlier investigations.

Table 1. Analysis of Control Hops and those Sprayed with Gibberellic Acid (after Kilning)

Sample	Date of sampling	Days after spraying	Nature of sample	Total soft resin (per cent) (ref. 3)	α-Acid/ polarimetric (per cent) (ref. 3)	Essential oil (per cent)
1	4.8.60	16	Control	1·3	0·00*	—
			Treated	1·7	0·05*	—
2	17·8·60	29	Control	1·3	0·04*	0·03
			Treated	3·3	0·27*	0·025
3	31.8.60	43	Control	13·8	6·7	0·42
			Treated	13·8	6·7	0·38
4	14.9.60	57	Control	13·3	5·4	0·65
			Treated	13·5	5·7	0·78
5	29·9·60	72	Control	13·6	6·3	0·86
			Treated	13·7	6·5	1·00

* By ion-exchange chromatography (ref. 2).

At harvest the control hops yielded 3·25 lb. of green hops per bine while the treated hops only gave 2·59 lb. of green hops per bine. Both groups contained the same percentage of dry matter (23·3 per cent), soft resin, and α-acid. These results are therefore in complete contrast to those of Nash and Mullaney. The differences in the results of the Australian and British investigations cannot as yet be accounted for with certainty, but may be due to varietal or perhaps climatic conditions. For example, during the period of this trial 10·22 in. of rain were recorded at Wye, compared with a 25-year average value for this period of 7·27 in. The hours of sunshine per day and the mean temperature were also lower than the 25-year average. In view of these circumstances it is desirable to repeat the trial during at least one further year and the results of the more extended investigations will be reported in full detail later. Nevertheless, the present results are of interest in establishing that the earlier Australian results do not necessarily reflect a general effect.

We thank Dr. A. H. Cook, Dr. H. S. Darling and Dr. G. Harris for their interest and advice in these investigations.

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Effect on Growth and Development

Gibberellic acid has been shown to increase markedly the rate of elongation and the length of internodes in many plants[1]. Several workers have shown that juvenile characteristics, notably leaf form, can be prolonged or induced using this substance[2–4].

The rate of elongation of the hop during the vegetative or spring phase of growth is very considerable for a temperate plant. Shoots appear above ground in April and may reach a height of five metres by the end of the first week in June. Mature plants can sustain daily elongation rates of 8–10 cm. during this period. The leaf-form of the earliest leaves is entire; those developing later show a transition from the simple to the three, five or seven lobed 'adult' form. In the course of a series of experiments it was decided to examine in the summer of 1958 the effect of gibberellic acid on shoot-growth and leaf-form during the vegetative phase.

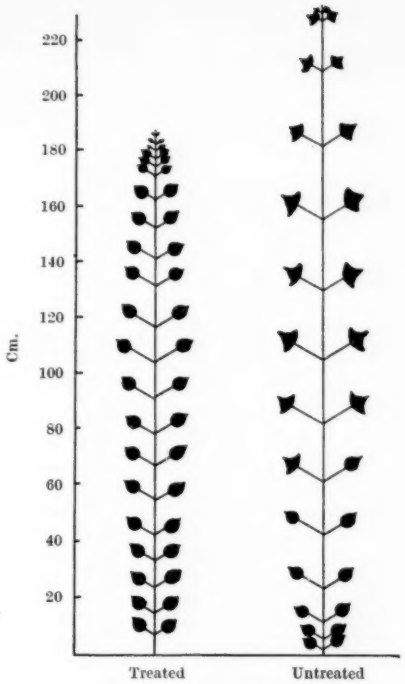

Fig. 1. Leaf-form and internode-length

Vernalized[5] seedlings were grown in a cool glasshouse. Treatment began immediately after the cotyledons had expanded. Gibberellic acid was applied, using a hand spray, at 5-day intervals at strengths of 1, 5 and 10 p.p.m. The treated seedlings elongated more rapidly initially, and the first two or three internodes were considerably longer than those of the controls. Thereafter the maximum internode-length of the treated plants did not exceed 14 cm. whereas the control plants developed successively longer internodes which reached a maximum length of 26 cm. when the experiment was concluded. The rate of elongation declined in the treated plants so that their mean height was significantly less than the untreated plants at the end of the experiment. The number of nodes developed by the treated plants was, however, a third greater than the controls. The treated leaves remained entire whereas all the untreated plants developed the typical lobed leaves before the ninth pair (Fig. 1).

It seems possible that the natural rate of elongation of the hop shoot, after the first few internodes have developed, represents the maximum capacity of cell elongation in this plant. In this case gibberellic acid may have had a secondary effect in speeding up the rate of meristematic development shown by

the greater number of nodes which the treated plants developed.

The treated plants had a larger total leaf area than the controls, but their leaves were chlorotic, being very pale green in colour. This may have indicated a lower photosynthetic capacity than the controls where the leaves were normal in colour, and may have been responsible for the gradual decline in the rate of elongation of the treated plants.

From a fundamental point of view the present results are of some interest, but from a practical point of view it has yet to be shown that gibberellic acid will be of use in the growth of this crop.

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CHROMATOGRAPHIC INVESTIGATION OF THE METHYL ALCOHOL/CHLOROFORM-SOLUBLE CONSTITUENTS OF HONEYBEE LARVAL FOODS

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THE lipid content of the food given to the queen larvæ (royal jelly: RJ) remains fairly constant during the time of the larval growth, while that of the food given to 3–5-day-old larvæ (modified drone and worker jelly: MDJ and MWJ respectively) decreases considerably in comparison with the food of 1–2-day-old larvæ (drone and worker jelly: DJ and WJ)[1,2]. Since the nutrition plays a decisive part in the differentiation of caste in the honeybee[3], an investigation of any change[4] or heterogeneity[5] in the constituents of the larval food is important.

The larval foods were collected and stored as reported earlier[4]. In order to correlate the components contributed to the food by the glands, the hypopharyngeal, thoracic and mandibular glands were dissected from the freshly emerged bees and the 6-day post-emergence nurse-bees and placed in methyl alcohol/chloroform (1 : 2) and stored at − 40° C. with other larval food until further use.

For the analysis, each sample of the larval food was thawed and stirred, and a 1-gm. aliquot withdrawn for extraction. The extraction was carried out at room temperature in an Erlenmeyer flask with 20 ml. methyl alcohol/chloroform (1 : 2) mixture as the solvent and 20 min. stirring with the magnetic stirrer. The homogenate was filtered through a sintered-glass filter under suction. The process was repeated once with 10 ml. of the solvent mixture to assure complete extraction. Assays showed that close to 22 per cent of the material was extracted. The extraction of the glands was done by homogenizing them in the same solvent with the Potter–Elvehjem homogenizer and filtering through a 0·45μ pore-size 'Millipore' filter disk.

The separation of the constituents was done by ascending chromatography using thin films of silicic acid on glass plates[6]. 50 μl. of the filtrate samples were analysed by using different proportions of methyl alcohol/chloroform. 29 per cent methyl alcohol in chloroform gave the best separation. After the solvent front had migrated about 7 in., the plates were removed and dried at room temperature. Fluorescent spots were observed under 3660 Å. and 2537 Å. wave-length ultra-violet light. Following the ultra-violet observation, the replicated plates were treated respectively with different colour reagents, which included *p*-anisidine, ninhydrin, iron chloride, iodine vapour and concentrated sulphuric acid at 130° C. for half an hour. A composite representation of the various treatments is shown in Fig. 1.

It is seen from Fig. 1 that there is considerable complexity of constituents in normal and modified jellies of all castes. Apparently there is no difference in the long-wave (faint spots) ultra-violet fluorescent spots of young and old royal jellies (YRJ and ORJ). MDJ and MWJ are similar, but have one component less than DJ and WJ. YRJ and ORJ have one fluorescent component more than DJ and WJ with similar migration pattern, while the pattern of the MDJ and MWJ is different. These fluorescent spots could be of pteridine type as indicated by Remboldt and Hanser[7]. The one spot migrating to the top has originated in the mandibular glands of the nurse-bee and may be 10-OH-Δ-2-decenoic acid[8]. With the short ultra-violet light (dark spots), an additional difference is seen in MDJ and MWJ as compared with other jellies.

The colour reactions also illustrate the complexity involved and indicate from which gland a particular compound is contributed to different jellies. A positive reaction with iron chloride (a green spot turning yellow) is exhibited by YRJ, ORJ, MDJ, MWJ and extracts from the hypopharyngeal glands of nurse-bees; it is not present in DJ and WJ. Ninhy-

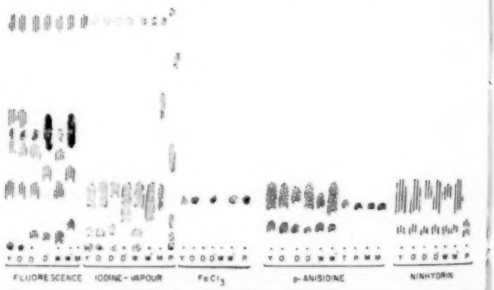

Fig. 1. Ascending chromatographic separation of honeybee larval foods and adult worker bee glands, revealed by various treatments on silicic acid plates
Y, young royal jelly (YRJ); O, old royal jelly (ORJ); D, drone jelly (DJ); D′, modified drone jelly (MDJ); W, worker jelly (WJ); W′, modified worker jelly (MWJ); M, mandibular glands of nurse bees; M′, mandibular glands of emerged bees; P, hypopharyngeal glands of nurse bees; T, thoracic glands of nurse bees

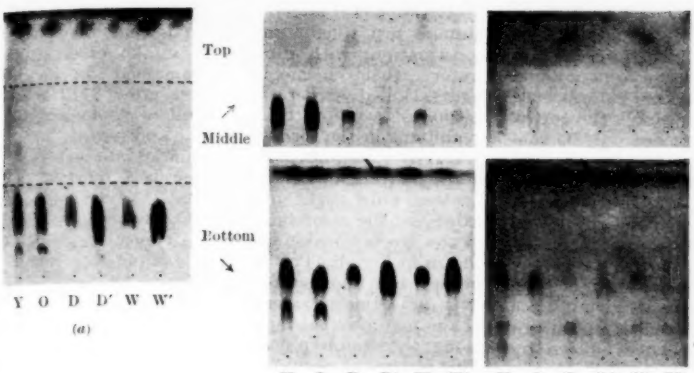

Fig. 2. Re-chromatographing of honeybee larval foods on silicic acid plates in different solvent systems. *a*, Solvent 29 per cent methyl alcohol in chloroform and sulphuric acid to char the constituents. The dotted line shows the divisions used for elution. *b*, Sulphuric acid charring. Upper photograph, middle division of (*a*) developed in 22 per cent methyl alcohol in chloroform; lower photograph, bottom division of (*a*) developed in 40 per cent methyl alcohol in chloroform. *c*, Iodine vapour treatment before charring. Upper and lower photographs correspond to upper and lower photographs of (*b*). Other abbreviations as in Fig. 1

drin- and *p*-anisidine-positive spots have similar migration patterns. The slower positive ninhydrin spots apparently originate in the hypopharyngeal glands of nurse-bees, while the migration of the *p*-anisidine-positive components from thoracic, mandibular and hypopharyngeal glands coincides with those fast-migrating spots of the jellies. The hypopharyngeal glands seem to contribute a larger share of *p*-anisidine-positive compounds. A preliminary paper-chromatographic separation shows that all the jellies have glucose and fructose, and sucrose is present in YRJ, ORJ, DJ and WJ while absent in MDJ and MWJ. On the other hand, MDJ and MWJ have maltose, which is absent in the rest of the samples.

The iodine vapour treatment indicating unsaturation exhibits four spots with a similar pattern in YRJ, ORJ, DJ and WJ, while there are only two components in MDJ and MWJ. These chromatograms were sprayed with concentrated sulphuric acid and heated to char the organic components (Fig. 2*a*). This treatment revealed other differences, but the resolution was not satisfactory. Better resolution was obtained by re-chromatography. Plates chromatographed with 29 per cent methyl alcohol in chloroform were divided into three as shown in Fig. 2*a*. The eluates from the top, middle and bottom sections were re-chromatographed in 5, 22 and 40 per cent methyl alcohol in chloroform, respectively. The top division did not reveal any significant qualitative differences as compared with the rest, which are shown in Fig. 2*b*. Quantitatively, the charring (Fig. 2*a*) in the top spots of YRJ, ORJ, DJ and WJ was at least twice that in MDJ and JWJ. This spot, as indicated earlier, may be 10-OH-Δ-2-decenoic acid. The concentration of re-spotted eluates in Fig. 2*b* at the point of origin was three times greater than that used earlier. The charring of the redeveloped plates was followed after the iodine treatment (Fig. 2*c*), so that the unsaturation could be compared with it. It is evident that the general pattern of the YRJ and ORJ remains the same and is not comparable with that of other jellies; however, there are some spots which are qualitatively similar though with quantitative differences. Unsaturation indicated by iodine vapour does not strictly correlate with the charring, particularly the slowly migrating components. This might be due to low concentrations of the components present and the amounts of reagents used to give coloured spots for visual observations.

A comparative study of the qualitative and quantitative changes of the components in relation to the age and different castes is of profound interest and importance. As is shown in this preliminary work, the biochemistry of the larval foods is quite complex. This complexity in turn is passed over to the nutritional phase and the phenomenon of dimorphism. The isolation, further characterization and incorporation of these biochemicals in a well-planned nutritional research investigation may help us to understand and control the formation of the queen and the workers.

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NERVOUS AND BIOCHEMICAL DISTURBANCES FOLLOWING HEXACHLOROBENZENE INTOXICATION

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OUTBREAKS of cutaneous porphyria, involving several thousand cases, have occurred in districts of south-eastern Turkey since 1956 [1]. Affected individuals, predominantly children, are said to exhibit photosensitivity, hepatomegaly and porphyrinuria but no abdominal or neurological symptoms. Poisoning by hexachlorobenzene, C_6Cl_6, introduced in bread made from dressed wheat intended for sowing, has been suspected as the cause of these disturbances. Ockner and Schmid [2] have confirmed such suspicions by administering hexachlorobenzene to adult male rats and observing neurological symptoms and

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increased urinary excretion of porphyrins and porphyrin precursors. Hepatomegaly with degeneration of liver cells was common in porphyric rats. There was no decrease in liver catalase or alteration in blood porphyrins or hæmoglobin, and no red fluorescence in the bone marrow *post mortem*, although the cortex of the long bones exhibited fluorescence. Of 33 rats started on the experimental diet, 13 died within the first month exhibiting terminal tremor, ataxia, weakness and paralysis but no major disturbance of porphyrin metabolism, while the remainder showed porphyrinuria, porphobilinogenuria and increased excretion of δ-aminolævulic acid after 2–8 weeks.

We have studied hexachlorobenzene intoxication in rabbits, guinea pigs, male and female rats and mice and have observed interesting differences in pattern of symptoms and chemical disturbances shown by each species. In addition, we have studied the susceptibility of the fœtal and new-born rat to poisoning from the hexachlorobenzene-fed mother. The biochemical picture seen in the rabbit most nearly resembles that described in man, and for this reason we have carried out most of our experiments with these animals. They are more rapidly affected by the poison and develop uroporphyrinuria, in contrast to the more usual coproporphyrinuria of the rat; disturbances of the central nervous system are relatively slight. Parke and Williams[3] found a single oral dose of hexachlorobenzene remained largely unchanged in the gut of rabbits after 5 days. There was no significant urinary or pulmonary excretion of metabolites. They found, however, increased urinary excretion of glucuronic acid, mainly in the free form. Cameron *et al.*[4] found subcutaneous injection of a dose as large as 0·5 gm. to rats produced no toxic effects.

Rabbits. Females (1·5–2·5 kgm.) were fed a diet prepared by powdering rabbit pellets ('Diet *S.G.* 1', Joseph Rank, Ltd.) incorporating 0·5 per cent of hexachlorobenzene, moistening with water, and then drying in shallow pans at about 60° C. Urine and fæces were collected separately. Five to six days were allowed for the establishment of basal excretion before institution of the drug. Pooled 2-day urines were analysed for uroporphyrin[5], coproporphyrin[6], porphobilinogen, δ-aminolævulic acid[7] and glucuronic acid[8]. Blood hæmoglobin and body-weight were recorded at the commencement and the end of the experiment. Tissue porphyrins[9] were determined after death. Organs were examined under ultra-violet light and those found to be fluorescent were prepared for ultra-violet microscopy. Paper chromatography was used for identification of the abnormal urinary constituents.

The general picture observed was development of increased urinary porphyrin, which was mainly uroporphyrin, after administration of the drug for about six weeks. This uroporphyrinuria became progressively more severe and was accompanied by a rise in coproporphyrin but a decline in glucuronic acid excretion, while there was no significant change in the levels of δ-aminolævulic acid or porphobilinogen. When determining the latter in the eluate from the 'Dowex 2' column[7], it was noticed that development of colour continued, reaching its maximum after about 6 hr. Chromatographic examination showed the substance responsible for this extra colour production to be not porphobilinogen but another Ehrlich reactor, possibly pyrrole-2-carboxylic acid. Rabbit urine is alkaline, and such a substance might be derived secondarily from a precursor (Gottschalk[10]). The identity of these materials and the relation they bear to administration either of the drug or of the heated diet (see later) are under investigation. The animals lost weight and suffered from oliguria and constipation, with diminished intake of food and water; their hæmoglobin remained unchanged. Unless killed earlier, they died after 8–12 weeks, having shown for the last week or so neurological symptoms in the form of tremor and paresis.

Out of 4 animals, 2 showed marked disturbance of porphyrin metabolism, another less marked, and another only slight disturbance. Nevertheless, all exhibited the same final neurological picture. Some of the biochemical changes in a typical example are shown in Fig. 1. Post-mortem examination revealed intense red fluorescence in ultra-violet light of the liver and adjacent tissue and of the bones, particularly of the metaphyseal region of the long bones but also along the diaphyphyseal insertion of the muscles. This picture of porphyrin distribution was confirmed by ultra-violet microscopy of suitably prepared transverse sections. There was no fluorescence in the bone marrow. The liver showed fatty change and patchy necrosis of varying degrees of severity. Under ultra-violet light, porphyrin was visible mainly in the centrilobular region. The degree of porphyrin fluorescence was related to the intensity of the porphyrin excretion during life. Necrosis, degeneration and focal calcification were seen in a few areas in muscles. No evident abnormality was found in the central or peripheral nervous systems in spite of the neurological symptoms shown by all animals.

Chemical analysis of organs showed massive quantities of a uro-type porphyrin in the liver, with only slight increase in the coproporphyrin and protoporphyrin fractions. Uro-type porphyrin was also detected in appreciable amounts in the serum and other organs examined. The qualitative reaction for porphobilinogen in the liver was negative. Paper chromatography of the uro fraction from the liver

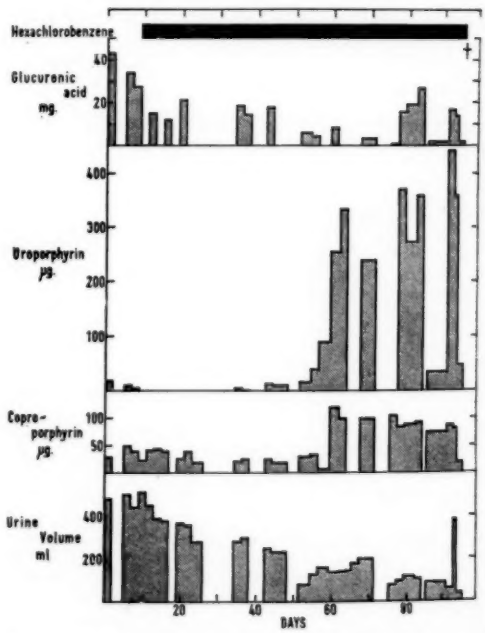

Fig. 1. Urinary volume and excretion of uroporphyrin, coproporphyrin and glucuronic acid of a female rabbit during hexachlorobenzene intoxication. Figures refer to 48-hr. periods

showed that it was mainly uroporphyrin III with some pseudo-uroporphyrin[11], whereas the uro fraction from the urine was predominantly pseudo-uroporphyrin. The urinary copro fraction was found to contain some coproporphyrin III together with pseudo-uroporphyrin and other porphyrins with chromatographic behaviour similar to hexa- and penta-carboxylic porphyrins.

Guinea pigs and mice. The level of dosage of hexachlorobenzene and means of administration in the diet were the same as for rabbits. These two species were found to be remarkably susceptible to hexachlorobenzene poisoning and to develop within 8–10 days very marked neurological symptoms. In guinea pigs, these took the form of continuous, rather coarse tremor of the extremities, interrupted by occasional clonic convulsions. There was also weakness, which was often more pronounced along one side of the body, so that the animals had difficulty in standing, could not right themselves if placed on the back and when moving in the cage they leaned against the wall for support. Mice exhibited a finer tremor involving the whole body, including head and tail. In place of convulsions, clonic contractions affecting mainly the hind limbs took place, so that the animals jumped like frogs. In both species there was a marked hyper-excitability, the symptoms being intensified by touch. If the drug was withdrawn within 24 hr. of development of nervous symptoms, recovery took place, but not otherwise. Death was preceded by progressive extreme weakness. All attempts to influence the symptoms described above by means of drugs such as hyoscine, chlorpromazine, phenylhydantoin, pyridoxine and asparagine were unsuccessful. Careful examination of the nervous systems of one guinea pig which died with typical tremor failed to reveal any structural changes.

Chemical analysis of the urine showed an early, marked increase in a δ-aminolævulic acid-like substance (see later), no significant changes being observed in porphobilinogen and porphyrin excretion except for a slight preterminal increase in coproporphyrin. Blood ammonia determinations were carried out in guinea pigs in view of the known ability of hexachlorobenzene to cause liver injury and the association of tremor and raised blood-ammonia in human cases of severe hepatic damage. All four guinea pigs so studied had ammonia values two to three times greater than their three normal controls. Liver histology, on the contrary, showed little evidence of damage apart from some fatty change.

Rats. The picture of hexachlorobenzene intoxication in rats resembles that in rabbits in its main features, with marked disturbance of porphyrin metabolism and only slight terminal neurological symptoms. The metabolic changes differed, however, in some important details. We observed a gradual increase in coproporphyrin and later of uroporphyrin with a terminal excretion of porphobilinogen which may be considerable. The identity of the latter was established by paper chromatography[12]; no chromogen reacting slowly with Ehrlich reagent was detected. The urinary coproporphyrin was identified chromatographically as mainly coproporphyrin III, only traces, if any, of the porphyrins intermediate between this and uroporphyrin being found. In addition to the picture described above, a prompt and sustained rise was seen in excretion of glucuronic acid and of a δ-aminolævulic acid-like material. The identity of the latter is still under investigation. Coincident

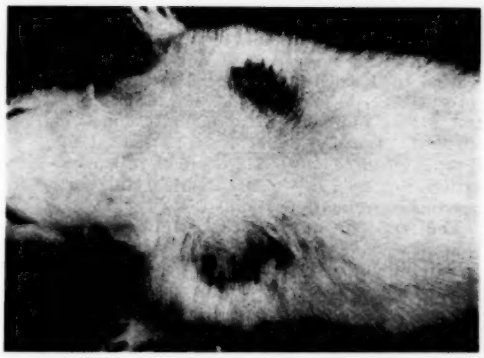

Fig. 2. Dermal lesions shown by a rat receiving hexachlorobenzene

with the terminal rise in porphobilinogen, there is an increased excretion of true δ-aminolævulic acid. Excretion of the δ-aminolævulic acid-like material and the rise in urinary glucuronic acid were both found to be associated with consumption of a diet prepared by heating and are therefore not relevant to intoxication by hexachlorobenzene.

In addition to the general picture of terminal neurological disturbance, some rats showed, at an earlier stage, cutaneous lesions in the form of depilated sores with hæmorrhagic crusts. These appeared before the animals developed porphyrinuria and were often symmetrically placed near the shoulder (Fig. 2). The animals showed signs of itching; hence scratching may have played some part in the development of these lesions. Quantitative tests for photosensitivity with a monochromator[13] were kindly carried out for us by Dr. H. A. Magnus but proved completely negative. Tests repeated during the phase of increased urinary porphyrin excretion failed to produce reaction at either 380, 400 or 420 mμ.

Two pregnant female rats were also given hexachlorobenzene in their food. One died, but the other was delivered normally and reared the young until they died with convulsions after 7–8 days. She was then given 3 normal week-old baby rats to foster. These died 3–4 days later with convulsions. The mother died 4 weeks later showing the usual picture. It would appear that the baby rat is particularly susceptible to the nervous effects of this poison.

The hexachlorobenzene used for the above experiments was the technical chemical, since examination had shown it to be free from less-highly chlorinated hydrocarbons. To eliminate the possibility that the observed toxic effects could be due to an impurity, some mice and rats were given pure hexachlorobenzene prepared as a standard for the estimation of halogen (British Drug Houses, Ltd.). These animals reacted in exactly the same way and after the same time as did those receiving the technical-grade product.

Discussion

Experiments with these four species of animal have confirmed the toxicity of hexachlorobenzene. Whereas cutaneous lesions dominate the picture in man and nervous symptoms are absent, our experimental animals have all shown striking neurological symptoms which were not associated with pathological change histologically evident in the central or peripheral nervous systems. It would therefore seem that these symptoms have a biochemical origin, and in this

connexion it is interesting that affected guinea pigs had raised blood ammonia.

The nervous symptoms do not appear to be closely related to disturbed porphyrin metabolism, since in susceptible species they develop relatively rapidly and before pigment metabolism becomes markedly affected. Porphyrinuria must be looked upon as a chronic effect of continued hexachlorobenzene intoxication, not seen in those animals dying earlier from nervous complications.

The experiments on rats confirm Ockner and Schmid's[2] report of increased urinary porphyrin and porphobilinogen excretion after prolonged administration of hexachlorobenzene. A rise in true δ-aminolævulic acid usually occurs coincidently with the rise in porphobilinogen towards the end of the experiment. The cutaneous lesions we have seen in this species suggest chronic dermal irritation rather than photosensitivity.

The disturbance caused by the drug in the porphyrin metabolism of rabbits was more marked than in the rat and also resembled the human condition more nearly. As in clinical material from Turkey, uroporphyrin was the predominant urinary porphyrin and porphobilinogen was consistently absent. Post-mortem analyses revealed massive deposits of uroporphyrin III in the liver, appreciable uroporphyrin in the plasma and several other organs, including bones, but no significant increase of porphyrin in the bone marrow. The porphyrin in the urine was mainly of uro-type, including pseudo-uroporphyrin; chromatography of the copro fraction revealed that even here pseudo-uroporphyrin and hexa- and penta-carboxylic porphyrins were strongly represented. Such a distribution within the body strongly suggests a porphyria of hepatic type with possible interference to the uroporphyrinogen decarboxylating mechanism.

Increase in the excretion of glucuronic acid was not observed in the experiments on rabbits, although this has been reported by Parke and Williams[3] to follow administration of hexachlorobenzene. Cutaneous lesions were not seen; our rabbits were housed in diffuse light, and no tests for photosensitivity were carried out.

Many problems remain to be solved in connexion with hexachlorobenzene intoxication. Investigation of the pathogenesis of the porphyrin dyscrasia in different animals should help to throw light on the condition caused by this drug in man. The somewhat surprising findings of neurological disturbances arising in animals, which might well be due to the relatively high dosage-level employed, are of intrinsic interest and deserve further investigation.

Dr. W. R. Lee, of the Institute of Orthopædics, kindly prepared and examined by ultra-violet microscopy the sections of porphyrin-containing bone from our rabbits. We wish to thank Dr. J. Smith for advice on the histological material and Mr. R. Belcher and Mrs. A. Latter for technical assistance. Dr. M. D. Turner of the Royal Free Hospital kindly carried out the determinations of blood ammonia.

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MECHANISM OF SENSITIVITY TO HYDROSTATIC PRESSURE IN THE PRAWN, *PALAEMONETES VARIANS* LEACH

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MANY marine animals exhibit sensitivity to hydrostatic pressures of less than 100 lb./in.^{2}; some being sensitive to pressures equivalent to a depth of only a few centimetres of water. An increase of pressure causes active swimming upwards, a decrease usually causes passive sinking, but some forms swim actively downwards[1-3]. The mechanism has so far remained obscure.

The prawn *Palaemonetes varians* is sensitive to pressure, as are a number of Crustacea, and if confined in a bottle it will swim to the surface when a pressure of 40 lb./in.^{2} is applied and will sink again when it is released. The surface potential on a prawn, together with the electrode potential, investigated crudely by passing one copper electrode into the abdomen and placing the other in the water beside it, was found to be 50 mV. or more negative to the surrounding salt water. When the electrodes were connected through a 50-ohm galvanometer, the flow of current proved to be sensitive to pressure; although it declined rapidly, repeatedly increasing the pressure to 40 lb./in.^{2} for a few seconds, caused corresponding increases of current by up to 20 per cent. Investigation of the electric field in the water surrounding a prawn free in a shallow dish, showed that the ventral surface is the more negative. Vital staining with nile blue showed that the negative parts stain deeply, namely, the gills and the part of the abdomen bathed by the water coming from them. The potentials arise partly from differences of *p*H in the water bathing the two electrodes.

Since different parts of the prawn differ in potential, and small currents flow, it seemed possible that sensitivity to pressure might reside in the properties of a very thin film of gas produced electrolytically on some surface. To see if this might be feasible, a model was constructed in the form of a diffusion cell, consisting of a 6·0-cm. length of 7 mm. glass tubing with one end open and the other closed by a 'Cellophane' membrane. The tube was filled with

10 per cent gelatine and strong salt solution, and was supported vertically in the pressure bottle half-full of 3·5 per cent salt solution with the 'Cellophane' membrane below the surface and the open end above. One copper electrode was passed into the gelatine, the other into the surrounding salt solution. A diffusion potential was set up, the inside of the cell being negative to the salt solution as in the prawn. Single cells did not prove very sensitive to pressure at first, although they became so on standing for a day. When charged by a slightly larger outside potential, a freshly made cell immediately became sensitive to pressure.

As the prawn has different potentials across different parts of its integument, it may be regarded as two cells connected in parallel, one charging the other. In a further model therefore, two cells similar to that discussed here were mounted side by side in the same bottle (Fig. 1), producing potentials of −60 mV. and −20 mV., respectively, relative to the solution by virtue of their different internal salt concentrations. On connecting the two central electrodes through a 50-ohm galvanometer, the current recorded at once became sensitive to pressure, being 5–10 per cent greater at 40 lb./in.². This arrangement (Fig. 2) represents the free prawn, where the inner parts of the body surface of differing potentials are connected electrically by body tissues, the outer by sea-water. The current between these cells and the surrounding solution, an arrangement representing the prawn mounted on one electrode, is also sensitive to pressure. Experiments showed that the sensitive element is the weaker cell, charged by the stronger. The electric currents are very small and no gas can be seen until some hours have elapsed. Gas is, however, produced electrolytically in small amounts down to zero potential on a metallic electrode[4], and we may conclude that such a thin gaseous film forms immediately on connecting up. The layer may be only a few angstrom units thick, insufficient to produce obvious optical effects, but nevertheless having the compressibility of a gas and producing electrical effects which vary with pressure.

The outstanding question is whether such a gas film can be formed on the surface of an animal.

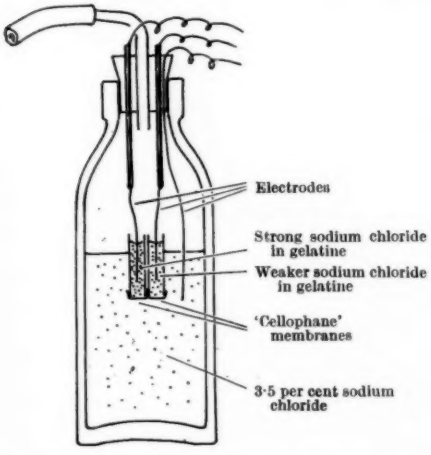

Fig. 1. Pressure bottle showing arrangement of two cells containing different concentrations of sodium chloride in 10 per cent gelatine

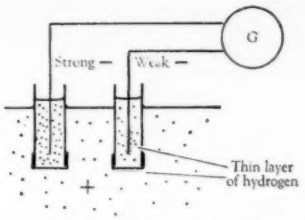

Fig. 2. Pressure-sensitive system, corresponding to arrangement in shrimp swimming free in water, when internal tissues take the place of the galvanometer

In order that gas shall be formed electrolytically, electron conductance must occur, as in the metallic electrode. That this is possible in the prawn may be demonstrated by applying a potential of 100 V. across the surface in distilled water, when hydrogen is freely evolved from much of the integument and, if a trace of copper sulphate is added, metallic copper is deposited. The hydrogen or copper ions are discharged by electrons passing through semi-conducting molecules or structures in sufficient numbers to produce visible amounts of discharge products at the high voltage. By the same procedure, hydrogen may be evolved from and copper deposited on a membrane of 'Cellophane' stained with methylene blue or coated with beeswax. At the lower voltages prevailing normally in the living animal, a correspondingly smaller number of ions will be discharged.

That such electrolytically produced gas layers occur in the prawn is suggested by the increase of sensitivity which follows the addition of salt and acid to the surrounding water. Further evidence is provided by experiments with surface-active substances that ionize to give charged complexes. Thus small amounts of 'Cetavlon' cetrimide B.P., which gives a negative bromide ion and a positive complex, completely destroyed the pressure-sensitivity of both the prawns and the diffusion cells. Small amounts of 'Teepol', which yields a negative complex, did not destroy this sensitivity in either, but sometimes enhanced it. As the positive complex which destroys the pressure sensitivity will migrate to the negative surface and there alter the charge, it is the most negative surface that is liable to blocking with 'Cetavlon' and is therefore first the gills. It is the outer surface which is coated because, after abolishing by 'Cetavlon' treatment, the sensitivity could be regained by immersing in sodium bicarbonate solution for some minutes to remove the substance, rinsing in acid water and transferring to fresh saline. The thin layers of hydrogen will, however, tend to be formed electrolytically on the outer surface wherever electrons can leak through the semiconducting structures in the integument under the influence of the normal potential drop across the surface.

Natural substances poison the surface, for prawns lose their sensitivity to pressure when crowded in a vessel with no seaweed and regain it when kept in a vessel containing seaweed. When lost, the sensitivity can also be restored by bicarbonate and acid rinses. Indeed the latter treatment, followed by the addition of a little salt, was found to induce or enhance sensitivity to pressure in the freshwater *Daphnia pulex*, which when newly caught showed only a trace of this reaction.

The widespread nature of charged surfaces in living biological material renders it likely that a capacity for pressure-sensitivity, based on the proper-

ties of a very thin film or perhaps partly adsorbed layer of electrolytically produced gas, may develop in a variety of situations where free hydrogen or hydroxyl ions are available against the surface of a charged membrane containing semi-conducting substances which allow a leakage of electrons.

Details of this work, which is continuing, will be published elsewhere. Certain initial experiments were carried out at the Kristineberg Zoological Station, Sweden; my thanks are due to the Director and his staff.

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LOCATION OF POLYGENES

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QUANTITATIVE genetics, hitherto, has been a subject distinct from Mendelian genetics in the sense that the specific genes segregating in any particular situation have not been handled as separate entities.

The first step of classical genetics is to identify the genes concerned as entities separable in heredity and to locate them on linkage maps so that thereafter they may be handled unequivocally in experiment. Our ability to do this depends on the classification of phenotypes into discrete groups, and the characteristic statistical techniques involved are those based on the χ^2 test of goodness of fit.

In the study of quantitative characters, on the other hand, the continuous nature of the relevant variation naturally leads to analyses of variance and, hence, to a biometrical approach. Using this approach has given us great insight into the problems, and we know that continuous and discontinuous heritable variation have in common the properties of segregation, dominance, interaction and linkage. It is established beyond doubt that the genes of biometrical genetics and the major genes of Mendelian genetics are subject to the same rules of inheritance for the same reasons; they are chromosomal[1].

Though the techniques of biometrical genetics are most elegant and informative, the resulting understanding of quantitative variation lacks the precision it would be given if we were to locate relevant genes with greater accuracy in linkage maps. Only when this has been done will we be able to look at a genic difference that has only been detected by its effect on a quantitative character and discover what it does and how it contributes to phenotypic variation.

The nature of the variation makes this difficult. In the best-studied examples, it is clear that the variation arises from genetic variation at a number of loci, and that the contribution of any locus to phenotypic variance is sufficiently small relative to that of the other loci and that arising from environmental causes and accidents of development to make the effects of that locus difficult to handle independently. To some extent this has affected our attitude to continuous variation, so that even those whose specific study is continuous variation, whether from a pure or applied point of view, seem rather often to act as if they thought that experimental control of the separate loci were in principle impossible. Statements occur in the literature such as "it is impossible to show the presence of these groups of linked polygenes by normal Mendelian methods"[2] or " . . . non-identifiability of genotypes and dependence on environment . . . have led to the development of a branch of genetics to which the name biometrical genetics is applied"[3]. Such statements may, however qualified by their authors, lead readily to a general feeling that any attempt to locate the genes of quantitative genetics would be defeated before it began. Those who are not specifically concerned with understanding quantitative genetics, but are interested in a character from some other point of view, may well be forgiven if they often seem to regard the demonstration that the character is inherited 'polygenically' as an end to investigation rather than a beginning.

Now it is clear that there is nothing in principle, though it may be difficult or laborious in practice, to prevent us from handling the genes concerned with quantitative variables by more or less classical genetic methods. Biometrical genetics is based on the fact that discontinuous variates, if there are enough of them, may be treated as if they were continuous variates, and it is implicit that the underlying genetic discontinuities are there to be uncovered. In fact, Johannson did just this by progeny testing and demonstrating that a number of different classes of inbred lines could be established from a strain varying in seed weight. Sax used a different approach when he used a marker to classify chromosomes and hence to detect linkage between major genes and quantitative genes[4], and the elegant experiments of Breese and Mather[4] go still further in this direction by splitting a chromosome into a number of component pieces and showing each of them to contain some of the relevant factors. But what has not been done is to exploit the possibilities fully by combining these two approaches.

My colleagues and I have had some success at this in the past few years, and it seems worth while to describe the basis of our techniques.

These depend upon the following simple principles: Suppose that we have found that a chromosome gives a higher value of a metric character than that given by a homologous chromosome marked with the recessive major genes *a* and *b*. We then test-cross individuals possessing this chromosome to the homozygous *ab*/*ab* stock, pick up equal numbers of the $++/ab$, ab/ab, $a+/ab$ and $+b/ab$ progeny and assay them for the metric character.

Let genes increasing the value of the metric character be *H* and their alleles *L*.

Suppose there is only one *HL* locus. Then the F_1 individuals test-crossed are either $H++/Lab$, $++H/abL$ or $+H+/aLb$ depending on the position of the *HL* locus in relation to the marker loci. Assuming the locus to be near enough to the markers for linkage to be detected, then the first two arrange-

ments will give results such that one recombinant marker class is similar to one of the parental classes, and the other recombinant marker class is similar to the other parental class. The $+H+/aLb$ arrangement will give different results. Ignoring double crossing-over, the $++$ class will all be H, the ab class will all be L. A proportion of the $+b$ class will be H, the rest L, and reciprocal proportions of the $a+$ class will be H and L. The parental classes will therefore have distinct means but, since they are homogeneous, low variances. The recombination classes, on the other hand, will, as they are heterogeneous, have relatively high variances, their means being intermediate between the parental classes.

In investigating a particular chromosome, our first step is to search for a pair of markers which produce such results, that is to say, are outside the HL genes we wish to locate.

Now such results do not indicate there is only one locus. Suppose there are two, both between the marker loci, and suppose them to be in coupling. Then the F_1 individuals test-crossed are $+HH+/aLLb$. Again ignoring double crossing-over, the $++$ parental class of progeny are all HH, and the ab class all LL. The parental classes are homogeneous and different in mean. The recombination class $+b$ may be either HH, HL or LL, and the recombination class $a+$ either HH, LH or LL. Again the recombination classes are heterogeneous, and will have intermediate means and high variances.

The essential problem is to distinguish between the one locus and the two locus results. This is relatively easy. With one locus each marker recombinant class falls into two classes; they are either H or L. With two loci each marker recombinant class comprises three classes, HH, HL or LH, and LL. If, then, we progeny-test adequately a sufficient number of each marker recombinant class to determine how many classes it contains, we can determine whether one or two (or more) loci must be invoked to explain the difference in which we are interested. If we proceed far enough, we shall be able to assess not only the number but also the frequencies of the classes and hence obtain map-distances.

The investigation of a particular quantitative genetic difference thus proceeds in the following way: We first investigate which chromosomes are effective, by test-crossing with a stock in which, if possible, each chromosome of the haploid set is marked, and in the same breeding programme separate from one another the chromosomes of the genome in which we are interested. We then test the effective chromosome against a number of marked stocks in order to determine which are suitable markers. We then obtain adequate numbers of recombinant chromosomes and classify them by progeny-testing to see whether they fall into two or into three classes.

The feeling that the genes of quantitative genetics may not be readily located has been strengthened by the knowledge that much of the relevant genetic variation is cryptic. It is to this that Kempthorne referred in using the phrase "unidentifiability of genotypes" in the quotation referred to here. Such a situation occurs when two genomes, or more particularly two homologous chromosomes, differ genetically, but not in their phenotypic effects, because the relevant genes are in the repulsion phase. But this does not make it impossible to handle the genes concerned in breeding experiments. Consider again two loci: the heterozygote is HL/LH and will produce HH and LL gametes the presence of which can be detected in the progeny of such heterozygotes, for they will have a higher variance than the progeny of individuals homozygous for either chromosome. It was in fact just such a test that led Gibson and Thoday[5,6] to discover and locate the relevant loci in the population in which they were interested.

More sophisticated techniques can be derived easily to break down more complex situations so that they can be treated in this simple way, to deal with homozygous as well as heterozygous effects of the chromosomes being analysed, and to re-synthesize the analysed chromosomes to check conclusions. When it proves necessary to use markers themselves affecting the character concerned or marker stocks themselves possessing inconvenient combinations of relevant polygenes, the elegant breeding system of Breese and Mather[4] can readily be modified to include the only essential of our method, sufficient progeny-testing of sufficient recombinant chromosomes of each marker class to determine how many classes they fall into in terms of their effect on the quantitative character.

The main practical limitation of the technique seems to be the availability of suitable markers, and the time that can be given to the considerable work involved. No doubt there is also a lower limit of genetic differences that would make genes of slight effect difficult to isolate, because there is a limit to the reduction of other sources of variance that can be achieved, though inbreeding of the marker stocks can help. The technique may therefore lead to isolation of non-random samples of polygenes of larger than average effect, can only tell us what is the minimum number of loci we must invoke, and may not tell us how complex the loci identified are. Whether these will prove serious limitations we do not know.

We have, in fact, had considerable success using such techniques in the analysis of sternopleural chæta number loci in *Drosophila melanogaster*. We have explained a major response to artificial selection in a line 'dp1', of Thoday and Boam[7] in terms of two loci at about 28 and 32 centi-Morgans on the third linkage group. Wolstenholme has analysed the high-chæta number flies of the polymorphic population 'D+' of Thoday and Boam[8] and found them to be distinguished by two factors at about 49 and 51 centi-Morgans on the third linkage map. Using these and also other techniques made possible by lethality interactions of the genes concerned, Gibson has located two factors from the low-chæta number flies of the 'D+' population at about 27 and 47 centi-Morgans on the second linkage group. In the latter case we have for the first time shown how the relevant loci occur in the population from which the selection line is derived[6]. We are at present analysing second chromosomes derived from the populations of Millicent and Thoday[9,10] and have roughly located two factors in these at about 47 and 64 centi-Morgans.

None of these investigations has yet been pushed to the extent where we have tried to determine the relative frequency of the different classes, and in only one have we good evidence that two and not more than two loci are concerned. Nevertheless, we have gone far enough in each investigation to satisfy ourselves that the location of the genes is not too difficult, and are able to place them in the second and third linkage maps as shown in Fig. 1.

The precision of these locations is not great. We know 1 and 2 to be separate, and that both are between *h* and *eyg*; we know 3 and 4 to be about 20

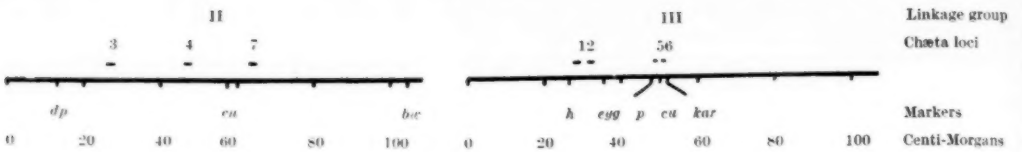

Fig. 1

centi-Morgans apart, that 3 is some 10 centi-Morgans to the right of *dp*, and 4 and some 8 centi-Morgans to the left of *cn*. We know 7 is to the right of *cn* and a long way from *bw*. We know that 5 is between *p* and *cu* and that 6 is to the right of *cu* and to the left of *kar*. The precision of the locations, and our knowledge of the chromosomes containing the relevant alleles are, however, sufficient for us to test future chromosomes against these and so identify new loci relatively easily.

Furthermore, we are now in a position to handle these loci in breeding experiments with sufficient precision to permit us to investigate their developmental effects. This should permit us to discover whether the different genes affect chæta number in different or similar ways. It may also permit us to discover other attributes of the various genotypes which are not so dependent on environmental and other sources of variance, and hence with some loci at least to classify genotypes directly from observations of phenotype that give discontinuity. It remains to be seen whether this will prove possible and if so whether it is only possible in special cases.

The present account of the principles of our methods is published with the view of encouraging others to approach their own problems in quantitative genetics with the possibility in mind of locating polygenes. An extensive attack on quantitative genetics made from this point of view as well as the biometric approach should be a great help in answering questions concerning the nature of polygenes, the randomness of their distribution in chromosomes, their relation to major genes and to heterochromatin, and whether or not they are a homogeneous or heterogeneous class of genes. At the same time, such an approach should bring new understanding of the consequences of artificial selection, and of the genetic architecture of natural and artificial populations.

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PREPARATION OF MITOCHONDRIA FROM THE PEEL OF APPLES

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TWO main difficulties in the isolation of active mitochondria from apple fruits are the low and well-buffered *p*H of the sap and the presence in the tissues of inhibitors, the chief of which has been thought to be of a polyphenolic nature, probably an oxidized *o*-dihydroxyphenol[1,2]. The successful isolation of mitochondria from the apple[3–5] has been achieved in recent years by the use of alkaline extraction media to neutralize the acid of the sap and by the use of peeled fruit, since the peel contains a relatively large amount of phenolic substances. The addition of ascorbic acid to the extraction medium has been advocated to keep these substances in the reduced form during the isolation procedures.

We are particularly interested in the metabolism of the peel of apples. Apart from the high concentration of phenolic substances in the peel[6] and their alleged effect on mitochondrial activity, peel tissue is difficult to disintegrate because of its leathery texture; satisfactory comminution cannot be achieved by grinding with sand in a mortar or by the use of a Waring blendor.

Grassman *et al.*[7] have shown that a polyamide material known as 'Perlon' or 'Ultramid' has a strong affinity for phenolic compounds, and Novikoff[8] has shown that the incorporation of polyvinylpyrrolidone (PVP) in the extraction medium preserves the morphological and biochemical integrity of mitochondria. Several workers[9–11] have advocated the use of PVP in the preparation of animal mitochondria, but its use has not been reported for mitochondria from plant sources.

A method has been developed for the preparation of mitochondria from the peel of Cox's Orange Pippin apples which incorporates the use of both 'Ultramid' and PVP and in which the tissue is disintegrated in a specially designed roller mill made in stainless steel. Briefly, the method is as follows: Peel (25 gm.) is added to a sucrose buffer solution (0·4 *M* sucrose) containing phosphate, citrate[12] ethylenediaminetetraacetic acid (EDTA) and 5 per cent PVP (molecular weight 25,000, pharmaceutical grade). The tissue is vacuum-infiltrated with the solution by placing in a vacuum desiccator for 10 min. and then releasing the vacuum. It is then passed through the diagonally serrated rollers of the mill and the ground material mixed thoroughly with the original solution to which has been added 5 gm. of 'Ultramid'. The tissue is then squeezed through two layers of muslin and the mitochondria separated from the filtrate according to the usual procedures for plant mitochondria. All preparative procedures are performed at 1° C. The particles are finally suspended in a solution of sucrose (0·3 *M*) and phosphate (0·2 *M*, *p*H 7·5) containing a small amount of adenosine triphosphate and adenosine monophosphate. These suspensions are used for assays of enzyme activity; uptake of oxygen and

Table 1. Succinic and Malic Oxidase Activity of Mitochondria from Various Sources

Source of mitochondria	Avocado		Apple				Sweet potato	Etiolated pea leaf	Tobacco leaf
Ref.	(14)	(15)	(3)	(4)	(5)	Present work	(2)	(16)	(12)
Oxidation measured at									
Temp. °C.	25	30	30	30	25	25	25	30	30
*p*H	7·1	7·1	7·4	7·0	7·1	7·5	7·1	7·3	7·1–7·3
Q_{O_2} (N) on substrate:									
Succinate	338	336	276	210	300	640	432	1,196	116
Malate	94	—	133	195	—	518	—	200	106

outputs of carbon dioxide are measured by the standard Warburg manometric methods at 25° C. and at *p*H 7·5.

In presenting a summary of preliminary results, it is felt that the special features of the method may be of importance, not only to studies of the mitochondria of fruits, but also to studies of plant mitochondria and other enzyme systems in general. The use of PVP may also have a wider importance in relation to any large molecules precipitated and 'inactivated' by 'tannins'.

More than 100 active preparations have been studied which stain with janus green and satisfy the criteria for Krebs cycle activity of mitochondria[12], including oxidative phosphorylation. Pyruvate is readily oxidized in presence of 'sparking' amounts of malic acid. The best preparations have succinic dehydrogenase activity higher than that of most other preparations of plant mitochondria (Table 1).

The effect of the incorporation of PVP in the extracting medium varies with *p*H ; and the uptake of oxygen and output of carbon dioxide to added succinate of preparations made with PVP over the range of *p*H 6·0–10·0 are shown in Fig. 1. It appears that these mitochondria are like those from other plant sources in that their activity is greatest when extraction is done at *p*H 7·0–7·5. Whereas in the absence of PVP the addition of ascorbic acid reduces darkening in the extracting medium,

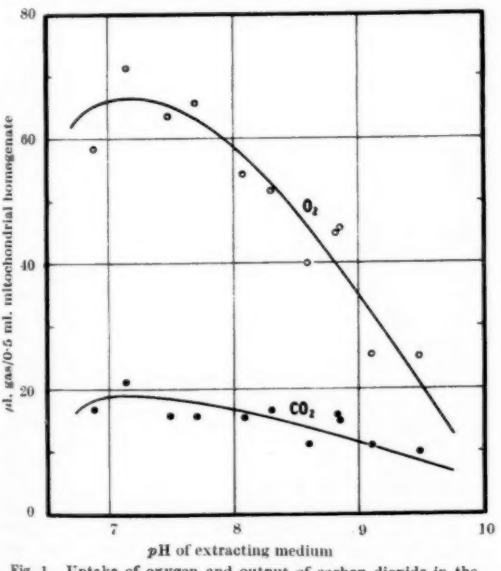

Fig. 1. Uptake of oxygen and output of carbon dioxide in the presence of 0·02 *M* succinate of mitochondria from apple peel prepared at various values of *p*H in the presence of PVP

it does not enhance the activity of the particles prepared with or without PVP. The incorporation of polyamide ('Ultramid') in the extracting medium does not enhance the activity of the particles, but it has the useful physical effect of coagulating a gel, which is otherwise troublesome during the first centrifugation.

At this stage the role of PVP must be largely a matter for speculation. It does not appear to be acting merely to protect (by complex formation) the mitochondria from inactivation by polyphenols. A range of phenolic compounds (chlorogenic acid, caffeic acid, phloridzin and *o*-coumaric acid) added to the Warburg flasks at a concentration of 0·001 *M* causes at the most a 10 per cent diminution in the activity of succinic oxidase; gallic acid at the same concentration brings about a 25 per cent inhibition, and catechol and tannic acid are the only compounds tried which cause complete inhibition. Tannic acid inhibition is partially reversed by adding PVP to Warburg contents. Even a fairly concentrated extract of the 'phenolic fraction' from the leaves of Cox's Orange Pippin apples causes only 35 per cent inhibition. Apart from catechol and tannic acid all these compounds produce a slight initial diminution in the output of carbon dioxide when succinic acid (0·02 *M*) is added. At higher concentrations (0·02 *M*) chlorogenic acid causes an uptake of oxygen about equal to succinate, and when the two are added together the uptake of oxygen is 85–90 per cent of that expected from simple additions of the separate uptakes, while the output of carbon dioxide due to succinate is unaffected.

Striking differences appear in the effect of the *p*H of the extracting medium, in the presence or absence of PVP, on the subsequent activity of the particles if the results are expressed on a nitrogen basis as compared with a given quantity of mitochondrial homogenate. Our methods are now sufficiently standardized and reproducible to justify this.

Results for preparations from comparable tissue are shown in Table 2. The rise in activity between *p*H 7·9 and 9·5 (extracting medium) without PVP, expressed on a nitrogen basis, agrees with the results of Lieberman[5] for mitochondria from apple pulp.

Table 2. μL. Oxygen-uptake (Activity) produced by Apple Peel Mitochondria prepared in Various Ways
(Average rate per hr. over first 2 hr. in Warburg)

Without PVP			With PVP		
Final *p*H of extract used	Activity per unit N of preparation	Activity per 0·5 ml. of preparation	Final *p*H of extract used	Activity per unit N of preparation	Activity per 0·5 ml. of preparation
6·6	80	23	6·8	295	57
7·5	85	36	7·5	365	70
7·9	73	32	8·1	330	55
9·0	145	26	8·9	255	38
9·5	210	38	9·5	162	25

but this rise is almost entirely a reflexion of the fall of the nitrogen content of the preparation over this range of *p*H. The nitrogen content of the particles prepared with PVP varies between 0·112 and 0·230 mgm./0·5 ml. of homogenate whereas without PVP the range is 0·177–0·500 mgm. nitrogen with all the values below 0·300 mgm. being above *p*H 8·0.

It is suggested: (1) The relatively high nitrogen content of particles prepared without PVP at values of *p*H between 6 and 8 is a result of the formation of insoluble complexes between non-mitochondrial protein and phenolic substances in the peel, these complexes being spun down with the mitochondria. (2) At *p*H values above 8–9 these complexes are soluble and are not therefore spun down with the particles. (3) PVP forms soluble complexes with the phenolic substances which cannot therefore precipitate non-mitochondrial protein to contaminate the mitochondria. Suggestions (1) and (3) are supported by the results of Guttram and Higuchi[17], who showed that phenols form complexes with PVP, and by Swain (private communication), who showed recently that tannin extracts form inactive complexes with certain enzymes, the inactivation being reversed by PVP and some other polymers.

Until the uncertainty as to the true mitochondrial-nitrogen content of our preparations has been cleared up, we prefer to express our results in terms of the amount of mitochondrial homogenate (Fig. 1).

The general higher activity of preparations obtained with PVP, regardless of nitrogen content, may also be due to the removal by the PVP of inhibitors, whether they be of a phenolic nature or not.

Full details of this work will be published elsewhere.

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AN AUSTRALOPITHECINE SCOOP MADE FROM A RIGHT AUSTRALOPITHECINE UPPER ARM BONE

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TWO years ago, I compared[1a] several antelope cannon-bone scoops from the basal grey breccia at the Makapansgat Limeworks site with a late Victorian sheep cannon-bone scoop (or apple-corer) made by a Ledbury lad in Herefordshire in the last decade of last century. Another scoop from the same grey breccia has now been found, but it was made from the distal end of an australopithecine humerus and by the different technique (see Fig. 1, extreme right specimen) of spiral splitting. For comparison with this australopithecine humeral scoop, the first figure includes a cast of the Ledbury apple-corer, a rather ornate apple-corer that came from the sale of an old Natal settler's effects and an imitation of the Ledbury specimen made from an equally slender antelope cannon-bone by Mr. Ronald Klomfass, technical assistant in the Anatomy Department of the University of the Witwatersrand. When found in a Johannesburg curio shop, the purpose of the Natal apple-scoop was unknown to its purchaser, Mr. J. C. Cohn; he found its counterpart eventually in a cannon-bone scoop in the collection of the Pitt-Rivers Museum at Oxford. Later, when he learned about the repetitive occurrence of antelope cannon-bone scoops in the Makapansgat basal grey breccia, he kindly gave me this Natal specimen. Presumably, it had been brought from England some time last century, possibly from Oxfordshire, by the original settler (whose name unfortunately is unknown).

Since the original announcement in *Nature*, much corroborative evidence has been forthcoming (ref. 2 and through newspaper reports as well as correspondence) about cannon-bone scoops. Spatulæ of this sort were in such general demand by our edentulous forefathers for pulping crisp fruits and eating other pulped foods that they were freely reproduced in ivory (or silver) for folk more well-to-do. These imitations were often divided into screw-threaded halves so that the scoop-half fitted into the handle-half for easy transport[2b]. The geographical and temporal gap between Victorian England and australopithecine South Africa also has been bridged considerably by the series of bone spatulæ (see Fig. 2) to which Dr. A. J. Arkell has kindly directed my attention[3]. Incidentally, this series shows that cannon-bone scoops were even more prized by ancient Egyptians than by recent Europeans. The left-hand specimen is "certainly fossil" according to Dr. Kenneth Oakley and possibly Palæolithic; the second is typical of several excavated by Dr. Arkell himself from the Neolithic site at Shaheinab near Khartoum in the Sudan. The other two (Nos. 3 and 4) were found among the grave furniture of two Egyptian tombs of the third millennium B.C.

The Victorian cannon-bone scoops were made with the aid of a pen-knife; the ivory imitations were turned on a lathe, as the Natal specimen may also have been. Mr. Trevor Jones of Salisbury, Southern Rhodesia, aided me in discovering the bone-on-bone

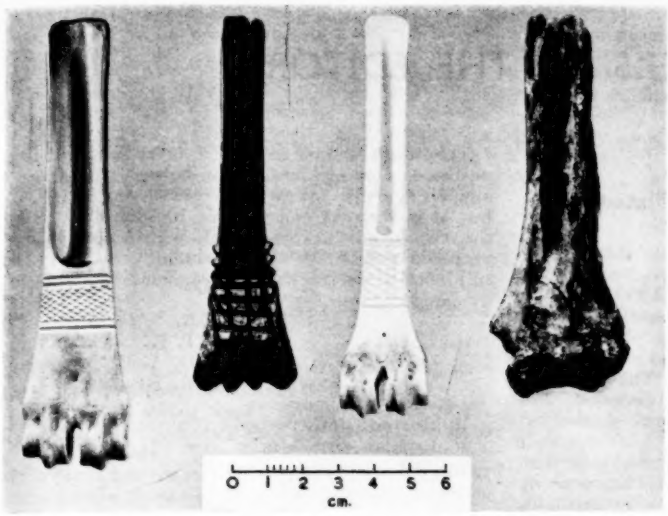

Fig. 1. An australopithecine and some recent bone scoops. The australopithecine humeral bone scoop from the Makapansgat grey breccia (extreme right) compared with —from left to right—a cast of the Ledbury bone scoop, the ornate Natal settler's specimen and an antelope cannon-bone scoop of similar calibre made recently in Johannesburg. Note the long spiral fracture of the australopithecine specimen and the abraded edges of the bone due to wear especially around its blade-like end

pounding or battering technique that produced the first australopithecine specimens described, and the precision labour it entailed[1].

This new australopithecine humeral scoop resembles more closely technically Dr. Arkell's cannon-bone spatulæ from the Nile Valley. It was prepared by the crack-and-twist technique, which was discovered by Mr. James W. Kitching and has been described elsewhere[4]. A single, well-controlled impact enough only to crack the bone enables one to twist the ends apart between the hands contrariwise.

Hazardous as it may appear to speculate from line-drawings how these Nile Valley bone spatulæ were prepared—especially when their surfaces have probably been smoothed by use, some possibly even on grindstones—the irregularity in the curvature of the broken bone surface of the Neolithic specimen (Fig. 2, No. 2) shows that it, at least, was first fractured by the same kind of crack-and-twist technique as *Australopithecus* employed at Makapansgat.

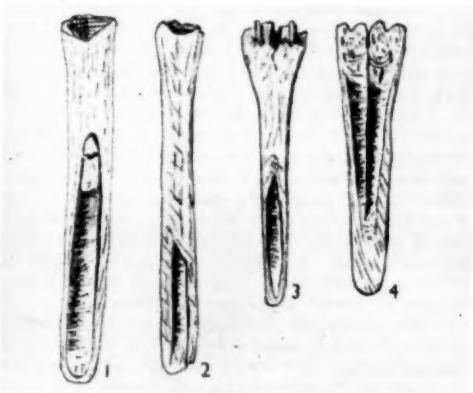

Fig. 2. Bone spatulæ from the Nile Valley (× 1/3). (1) A fossil (?) Palæolithic bone scoop from the Qua Badari district, Middle Egypt. (2) Typical cannon-bone scoop from Neolithic site of Shaheinab, near Khartoum. (3) and (4) Cannon-bone scoops found among the grave furniture of two Egyptian tombs of the third millennium B.C.

This crack-and-twist technique must also have been used by *Sinanthropus* in China, to produce the spiral humeral fractures of antelope and other bovid bones at Choukoutien, by Kalkbank man in South Africa and by Mesolithic man in Portugal[2,5]. So this early human bone-tool-making technique became dispersed over the entire Old World mass and has persisted from australopithecine times, when it was apparently invented, until very recently indeed.

This particular australopithecine scoop prepared from an australopithecine humerus also supports the inference made from the bowl-shaped condition in which the first female australopithecine occiput was found that *Australopithecus prometheus* was cannibalistic. The fact that it is the distal portion of a right humerus suggests that already some concept may have germinated in its fabricator's mentality that by its use he embodied some of his defunct opponent's vitality.

The value of the cannon-bone scoops or spatulæ lies not merely in the age-long persistence of the tradition they display but also in their overcoming the dental infirmities of mankind, for which they compensated. They explain the dental knives and scrapers and the food-pulping pestles and mortars of bone as well as the skull-bowls that accompany them. Upon these osteodontokeratic utensils domestic human family life was being built untold thousands of years before stone tools were adapted to its improvement. Von Koenigswald[6] has traced the human use of skull-bowls from *Sinanthropus* to Ngandong and recent times.

The interest of this australopithecine humeral scoop, like that of the australopithecine occipital skull-bowl, lies in the antiquity they reveal for human veneration of human bones above all other bones.

Acknowledgment is made to the South African Council for Scientific and Industrial Research, the Council of the University of the Witwatersrand, the Wenner-Gren Foundation and especially to the Wilkie Foundation of Des Plaines, Illinois, U.S.A., for the financial assistance which has maintained the field and laboratory work that has led to this and previous discoveries.

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LETTERS TO THE EDITORS

PHYSICS

Absorption Spectrum of γ-Irradiated Lead Silicate Glass

In an earlier communication[1] it was stated that the absorption spectrum of γ-irradiated lead silicate glass showed three absorption bands at 1·7 eV., 2·3 eV. and 3·3 eV. respectively. The separation of the spectral curve into these bands was obtained by assuming that the peak at 3·3 eV. was uninfluenced by the presence of any other bands. The points in the vicinity of this peak were used to estimate the first Gaussian and the other bands were obtained by successive subtraction.

As the amount of available data increased, further work employing different methods has been done in order to confirm this separation, making only the assumption that the spectral absorption curve consists of a small number of Gaussian-shaped components.

This problem is well suited to solution by means of an electronic digital computer and a programme was written using the equation:

$$y = \Sigma A_i \exp B_i (x - c_i)^2$$

where A_i corresponds to the peak height of the absorption band at a wave-length of $1/c_i$, B_i is a function of the half-peak width of this band, and x is the reciprocal of the wave-length at the point considered. By a process of successive iteration it was found that the full spectral absorption curve in the region 350–1,000 mμ could be accurately represented as the sum of two Gaussian absorption bands with maxima at 2·54 eV. and 3·36 eV.

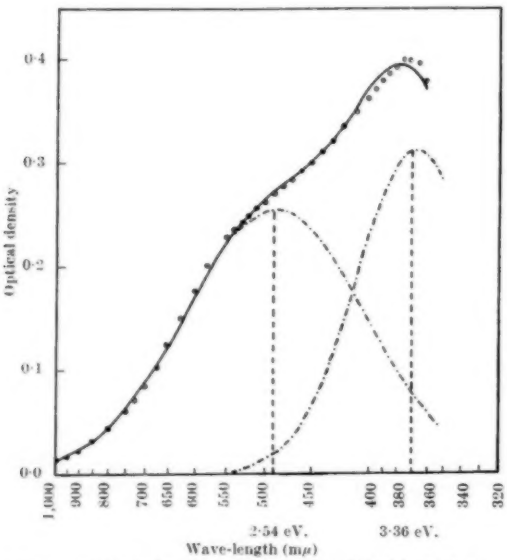

Fig. 1. Spectral absorption curve for γ-irradiated lead silicate glass. Dose, 3·2 × 10⁴ rads after 455 min.

The result shown in Fig. 1 is typical of many results so obtained, and it can be seen that there is now no evidence for an absorption band at 1·7 eV., but that there is considerable interaction between the two bands at their peak wave-lengths. The encircled points are the measured values and the full-line is the sum of the two Gaussian bands obtained by computation.

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Rotational Analysis of Absorption Bands of Lead Monoxide

The low-lying states of N_2 and of CO arise from electron configurations[1] which may be written:

$$\pi^4\sigma^2,\ X^1\Sigma^+ \quad (1)$$

$$\pi^4\sigma\pi,\ {}^3\Pi_r,\ {}^1\Pi \quad (2)$$

$$\pi^3\sigma^2\pi,\ {}^1\Sigma^+,\ {}^1\Sigma^-,\ {}^3\Sigma^+,\ {}^3\Sigma^-,\ {}^1\Delta,\ {}^3\Delta \quad (3)$$

In the group of molecules from CO to PbTe, the allowed absorption bands of longest wave-length may then be expected to be ${}^1\Sigma^-,\ {}^1\pi - X^1\Sigma^+$. This is found to be the case, for example, in SiS, where the systems $D^1\Pi - X^1\Sigma^-$ and $E^1\Sigma^+ - X^1\Sigma^+$ appear readily in absorption[2,3]. In addition, evidence about some other predicted states, notably $e^3\Sigma^-$, $I^1\Sigma^-$ and (?)$^1\Delta$, has been obtained from work[4,5] on the perturbations in $D^1\Pi$. In SiO, GeO and SnO there is no further strong absorption[6] until wave-lengths considerably shorter than those corresponding to the $E - X$ systems, indicating that more highly excited orbitals than (2) or (3) are here involved.

This comparatively simple situation, which obtains for the lighter molecules of the group, is in strong contrast with that for the heavier molecules, particularly for SnSe, SnTe and the lead compounds. In PbO, for example, the absorption region of longer wave-length (λ > 2500 Å.) shows at least six band systems[7]. The characters of the upper states of these systems have now been determined by rotational analysis of several bands of each system from plates taken on a 6·5-m. grating spectrograph. A summary of the results is given in Table 1. The results for the $A - X$ system agree with those from the less-extensive analysis of Christy and Bloomenthal[8]; however, their analysis of the $D - X$ system, which indicated that state D was $^1\Sigma^+(O^+)$ is incorrect.

In interpreting these results, two possibilities arise: either (i) new low-lying electron configurations other than (2) and (3) become of importance in the heavier molecules, or (ii) the coupling shifts to Hund's case-*c*. The latter is perhaps the more attractive suggestion. The case-*c* states of (2) and (3) are as follows :

$$\pi^4\sigma\pi^* \rightarrow 2,1,1,0^+,0^- \quad (2)$$

$$\pi^3\sigma^2\pi^* \rightarrow 3,2,2,1,1,1,0^+,0^+,\ 0^-,0^- \quad (3)$$

Table 1. SUMMARY OF CONSTANTS FOR ^{208}PbO

State		T_e	ω_e	$\chi_e\omega_e$	B_e	$10^5\,\alpha_e$	r_e Å.	Notes
E	0^+	34680	[440]	—	[0·2382]	[1·4]	$2·18_3$	1
D	1	30464	530·5	2·92	0·2711	3·1	2·046	2
C'	1	25720	[475]	—	0·248	1·8	2·14	2, 3
C	0^+	24108	[534]	[3·9]	0·254	2	2·11	4
B	1	22531	$493·4_7$	2·26	$0·2646_3$	2·6	$2·070_9$	2
a	?2							2
A	0^+	20088	451·7	3·33	$0·2586_9$	$1·3_4$	$2·095_2$	
X	0^+	0	721·8	3·70	$0·3073_7$	$2·1_2$	$1·921_4$	

(1) There are strong perturbations in state E, so that the constants for this state are uncertain. The values of v' for this system have been increased by 1, following a study of the isotope effect.

(2) Perturbations in R, P and Q branches at about the same value of J' are observed in some of the levels of B, C' and D. The form of these suggests that they are caused by one or more $\Omega = 2$ states.

(3) The system $C' - X$ was observed by Howell (ref. 9) as a single $v'' = 0$ progression.

(4) The values of v' for the $C - X$ system have been increased by 2, following a study of the isotope effect.

If these are all bound states, there are predicted three absorption systems $0^+ \leftarrow X$ and five systems $1 \leftarrow X$. The 0^- and 2 states would be identifiable in the absorption spectrum only through interactions with the $\Omega = 1$ states. The number and the character of the excited states so far observed suggest that their interpretation as case-c states may be the correct one. It is hoped that work, now in progress, of the numerous perturbations in these systems will help to elucidate this problem.

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Kirkendall Effect in Gases

AN experiment has been described[1] in which the motion of fine particles of ammonium chloride smoke in a diffusing gas mixture was considered evidence for a Kirkendall effect in gases. Only a rough semi-quantitative interpretation could be given at the time in terms of the usual phenomenological theory of the Kirkendall effect. Subsequent theoretical and experimental work[2,3] on gaseous diffusion has shown that the phenomenological theory cannot be correct for gases, but that the classical kinetic theory of gases is capable of accounting for the phenomenon quantitatively. We wish to point out that it is now possible to give a quantitative interpretation to the previous measurements.

The experimental results may be summarized in terms of the usual intrinsic diffusion coefficients. The observed motion of the finest particles of the smoke corresponded to a difference of intrinsic diffusion coefficients of $(D_2 - D_1) \simeq 0·63$ cm.²/sec. for $1 = CF_2Cl_2$ and $2 = H_2$ at 20° C. and 1 atm., in the absence of gravity. This value is probably an upper limit, since the necessary correction for the rate of sedimentation of the smoke particles under gravity appeared to be somewhat too large as it was made.

If the smoke particles are much larger in diameter than the mean free path of the gas molecules, they should drift with the bulk velocity (that is, the mass average velocity) of the diffusing gas mixture and thus act as true Kirkendall markers. In this case the difference of the intrinsic diffusion coefficients is given by equation (23) of reference 2, and can be written as:

$$D_2 - D_1 = \frac{m_1 - m_2}{x_1 m_1 + x_2 m_2} D_{12} \qquad (1)$$

where the m's are the molecular masses, the x's are mole fractions, and D_{12} is the interdiffusion coefficient, which has been found[3] to have a value of 0·38 cm.²/sec. Taking $x_1 = x_2 = 1/2$ (at the diffusion boundary), we calculate from (1) that $(D_2 - D_1) = 0·73$ cm.²/sec. This is larger than the experimentally observed upper limit of 0·63 cm.²/sec., so the smoke particles cannot be regarded as true Kirkendall markers.

If, however, the smoke particles are smaller in diameter than the mean free path, their behaviour is different, and the theory for such a case has been given by Waldmann[4]. According to his equation (7.20), the particles move with a velocity corresponding to:

$$D_2 - D_1 = \frac{m_1^{1/2} - m_2^{1/2}}{x_1 m_1^{1/2} + x_2 m_2^{1/2}} D_{12} \qquad (2)$$

from which we calculate $(D_2 - D_1) = 0·59$ cm.²/sec. This is in good agreement with the observed upper limit. In equation (2) we have assumed that Waldmann's coefficients of diffuse scattering of the gas molecules from the smoke particles were equal to unity, which is in accord with experiment[5]. It is apparent that the finest smoke particles are probably smaller than the mean free path. This is physically reasonable, since the relevant mean free path in the experiments was of the order of 0·1μ.

Although the smoke particles were thus not true Kirkendall markers, they could have been used as such, since their velocity was proportional to the bulk velocity, although not equal to it. From equations (1) and (2) the relation is:

$$v = \left[\frac{m_1 - m_2}{m_1^{1/2} - m_2^{1/2}}\right]\left[\frac{x_1 m_1^{1/2} + x_2 m_2^{1/2}}{x_1 m_1 + x_2 m_2}\right] v(\text{smoke}) \qquad (3)$$

or:

$$v = [1 + 2(m_1 m_2)^{1/2}/(m_1 + m_2)]\, v(\text{smoke}), \quad \text{if } x_1 = x_2 \qquad (4)$$

where v is the mass average (bulk) velocity and v(smoke) is the velocity of the smoke particles.

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ENGINEERING

Effective Contact Stresses and Friction

The earliest statement concerning statical frictional forces between rigid bodies is usually attributed to Coulomb (about 1776). The principle of Coulombic friction is that the force (F) required to generate translation of one body relative to another is directly proportional to the external force (W) transmitted across the contact plane, so that:

$$F = \mu W \tag{1}$$

where μ is the coefficient of (static) friction.

In 1950, Bowden and Tabor[1] published their, now well-established, concept of asperity contact giving rise to plastic junction. According to Bowden and Tabor the frictional force (F) is a product of the total area of the junctions (Aj) and their shear strength (Sj), hence:

$$F = AjSj \tag{2}$$

and it was further postulated that the area (A) is given by the normal force (W) divided by the flow pressure (p) so that:

$$W = pAj \tag{3}$$

and thus :

$$\mu = \frac{F}{W} = \frac{Sj}{p} \tag{4}$$

and it has been found that for most metals the coefficient μ is independent of the normal load.

The purpose of this communication is to direct further attention to equation (3).

Terzaghi[2] was the first to state the law of effective stress in relation to inter-particle contact in a two-phase medium. Terzaghi's effective stress law states that the effective stress (σ^1) is given by:

$$\sigma^1 = (\sigma - u) \tag{5}$$

where σ is the applied (external) normal stress and u is the positive hydrostatic pressure in the fluid surrounding the 'solid' particles.

This law entails two basic assumptions: (i) that a continuous surface exists which passes through the contacts between particles; (ii) that the area of contacts (plastic junctions) is insignificant compared with the total area of the continuous surface. Experimental work in the field of soil mechanics has established the validity of this law within practical limits[3].

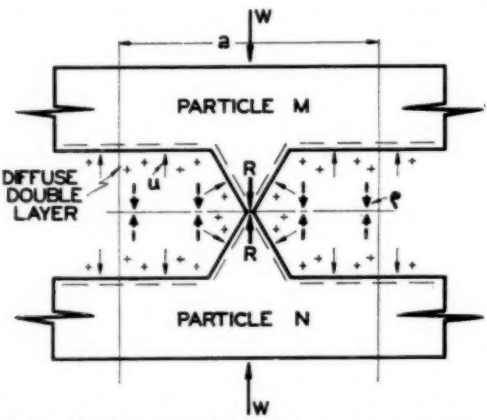

Fig. 1. Effective stresses at a contact. $a = A/N$ = effective area per contact; N, No. of contacts

Following a study of clay structure during recent years[4] I have extended equation (5) to take account of surface activity between proximate particles, and the effective stress equation now takes the form:

$$\sigma^1 = (\sigma - u + \rho) \tag{6}$$

where ρ is the effective stress component derived from surface forces.

Consider the simplified system shown in Fig. 1.

The force R transmitted across the junction is made up of three parts.

$$R = W - \mu.a. + \rho.a. \tag{7}$$

assuming that ρ, the resultant of all surface physico-chemical effects (for example, Van der Waals forces, double-layer repulsions, primary or secondary valence bonds at the junction), is attractive in character.

Then according to the Bowden–Tabor concept the frictional force (Fj) required to shear the junction is:

$$Fj = \mu R$$

thus:

$$Fj = \left(\frac{W}{N} - u.a. + \rho.a.\right) \tag{8}$$
$$= \frac{W}{N} - \frac{uA}{N} + \rho\frac{A}{N}$$

The total frictional or shear force (F) is then given by:

$$F = \sum_{j=1}^{N} Fj = \sum_{j=1}^{N} \mu R = N\mu R$$

It follows that the shear stress (S) is:

$$S = \frac{F}{A} = \frac{N}{A}\mu R$$
$$= \frac{N}{A}\mu\left(\frac{W}{N} - \frac{uA}{N} + \rho\frac{A}{N}\right)$$
$$= \mu(\sigma - u = \rho) \tag{9}$$

that is, the shear stress, on the basis of the assumptions given, is independent of the number of asperities.

Equation (9) is the effective stress expression governing friction in pure systems, that is, those where the junctions are all physically and chemically alike.

For composite materials, such as soils, the μ term is conventionally replaced by the term $\tan\varphi_e$ (Hvorslev, 1957), where φ_e is called the angle of internal friction or alternatively $\tan\varphi_e$ is the co-efficient of macro-fraction and is a statistical mean of the component micro-friction effects[5].

Although equation (9) was derived with clay-water-electrolyte systems principally in mind it is thought to be of more general application. For example, if both u and ρ are effectively zero we have the condition of Coulombic friction applicable to dry granular materials; at the other end of the scale we may have conditions where both σ and u are insignificant compared with ρ, as with the internal structure of metals and certain adhesives. This latter concept may even be extrapolated to the inter-atomic level in the solid state.

Yet another possible application concerns lubrication. If, for the present, viscosity effects are neglected, then for invert surfaces ($\rho = 0$) the condition of 'dry' friction is obtained when $\mu = 0$, even though fluid may be present and the necessary condition for hydrodynamic friction is that $\sigma = u$. Between these

two extremes there is a range of u values, the actual value of u and therefore that of the apparent coefficient of friction being dependent on the ambient conditions.

This latter approach is at present being considered in a study of high-speed skidding characteristics in relation to airfield pavements.

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CHEMISTRY

Quantitative Estimation of Peak Areas in Gas-Liquid Chromatography

QUANTITATIVE estimation of peak areas on gas-liquid chromatograms has been carried out by a variety of methods, which include triangulation, cutting out the peak areas and weighing the cut paper, planimeter tracing and use of an integrator built into the recorder. A new method recently proposed by Bartlet and Smith[1] is based on evidence that the peaks obtained with a well-designed chromatograph closely approximate a normal or Gaussian distribution curve.

The equation for this type of curve is given as :

$$Y = \frac{A}{\sigma\sqrt{2\pi}}\, e^{-\frac{1}{2}(X/\sigma)^2} \qquad (1)$$

where Y is the height of the curve measured at a distance x from the mean. A is the area and σ is the standard deviation. At the mean or central peak of the curve $x = 0$, $Y = h$ or peak height and equation (1) becomes :

$$h = \frac{A}{\sigma\sqrt{2\pi}} \qquad (2)$$

$$\text{or } A = 2{\cdot}507\, h\sigma \qquad (3)$$

The peak height h may be measured directly from the chromatogram and the standard deviation σ may be obtained from the peak width w at various heights since $w = \sigma$ at $0{\cdot}882h$, $w = 2\sigma$ at $0{\cdot}607h$, $w = 3\sigma$ at $0{\cdot}324h$ and $w = 4\sigma$ at $0{\cdot}134h$. The area under the curve may therefore be calculated from equation (3).

Bartlet and Smith also showed that σ is a linear function of the retention time t_R, and this has been confirmed in our studies with fatty acid esters. It is thus evident that the product of peak height and retention time may also be used as a measure of peak area. This is much better than using the product of peak height and standard deviation, since retention times are normally measured in any event and can be obtained with greater ease and precision than the standard deviations of the peaks.

The product of peak height and retention time was suggested by Pecsok as a measure of peak area[2], but no theoretical or experimental proof of its validity was provided and it does not seem to have been used very widely. The method was therefore compared in our Laboratory with the paper weighing and triangulation methods, using chromatograms of mixtures of fatty acid esters. Examples are shown in Table 1. Although the results obtained with the new method are not in perfect agreement with those of the other methods, they are nevertheless adequate for most biological studies, and the convenience of using the new method makes it very useful for routine analytical work.

In applying this method to peaks which are only partially resolved, it should be noted that the measured peak heights will be higher than the true peak heights by amounts which are determined by the degree of overlap and by the relative amounts of the components. A correction for this may be obtained by application of equation (1) as shown by Bartlet and Smith. Alternatively, if pure samples of the overlapping components are available, they may be chromatographed individually and correction factors obtained by direct measurement. In practice, the correction factors are very small, even for peaks with serious overlap, and although successive corrections are theoretically required, they are rarely, if ever, needed.

The present method of determining peak areas has an advantage over the paper-weighing method in that it allows the chromatogram to be saved for future reference. It is less laborious than triangulation and gives more reproducible results since it avoids the subjective variation involved in the drawing of triangles. It may also be noted that the units of measurement are unimportant as long as the same

Table 1. QUANTITATIVE ANALYSIS OF FATTY ACID ESTERS

Fatty acid methyl esters	Shorthand designation	Weighing method: Wt. of cut-out area (mgm.)	Weighing method: per cent	Triangulation method: ½ height × base (mm.²)	Triangulation method: per cent	Present method: Peak height (h) (mm.)	Present method: Retention time (t_R) (min.)	Present method: per cent
Palmitate	(16 : 0)	27·3	14·8	504	15·5	95	5·8	14·5
Oleate	(18 : 1)	49·5	26·8	900	27·7	88·5	12·2	28·4
Linolenate	(18 : 3)	69·7	37·7	1215	37·4	78	18·9	38·8
Behenate	(22 : 0)	38·3	20·7	630	19·4	17·4	39·8	18·2
Palmitate	(16 : 0)	33·8	15·0	635	15·7	114	5·8	15·1
Stearate	(18 : 0)	75·2	33·3	1341	33·1	128	11·0	32·1
Linoleate	(18 : 2)	76·1	33·7	1392	34·4	105·5	14·8	35·5
Erucate	(22 : 1)	40·5	18·0	680	16·8	17·5	43·5	17·3
From corn oil*	(16 : 0)	25·6	22·6	440	21·9	92·5	5·0	21·7
	(18 : 1)	45·6	40·3	805	40·1	80·5	10·5	39·7
	(18 : 2)	42·0	37·1	763	38·0	64·2	12·8	38·6
From rat plasma triglycerides*	(16 : 0)	48·3	44·6	885	45·0	162·5	5·0	44·7
	(18 : 1)	42·0	38·8	752	38·3	67	10·4	38·3
	(18 : 2)	18·0	16·6	328	16·7	24·5	12·6	17·0

Fatty acid esters were separated on a 6 ft. column packed with butanediol succinate (Craig ester) on Chromosorb *W* (1 : 4). A Barber–Colman chromatograph with a radium source for the ionization detector was employed. The column was operated at 200° and the flash heater and cell bath at 255°. Argon at 20 lb./in.² flowed at the rate of 150 ml./min.
* Minor peaks were not included in these calculations.

units are used for each component of the mixture. Thus, in the examples given, peak heights were measured in mm. and retention times in minutes. The convenience of this method and the probability that further improvements in instrumentation will give curves more closely approaching the Gaussian distribution curve suggest it as a method of choice for future quantitative work in gas-liquid chromatography.

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Compressibility and Specific Evaporation Resistance of the Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers

The monolayers of long-chain compounds such as cetyl and stearyl alcohols are being used extensively to minimize the loss of water by evaporation in reservoirs[1]. Permeability of water vapour through a monolayer depends on, among others, the magnitude of the van der Waals type of cohesive forces of the film molecule with its neighbours, and on the adhesive force between the monolayer and the water sub-phase. Further extension of the *n*-alkyl chain and/or suitable modification of the terminal polar group would, therefore, make a monolayer more effective in reducing evaporation.

In view of various such considerations as structural, stereochemical and spreading property, and economic availability, we have been restricted to the C_{16}–C_{22} *n*-alkyl compounds and prompted to modify and thus increase the interaction of the polar end-group with the water sub-phase. Accordingly, we synthesized a series of glycol monoalkyl ethers, R–OCH_2CH_2OH (R being an *n*-alkyl chain containing 16–22 carbon atoms), and reported[2] their superior water evaporation resistance behaviour compared to the alcohols. This communication describes the preliminary results on the compressibilities and the specific evaporation resistances of the monolayers of these as well as those of the alcohols for comparison.

The preparation and purification procedures of the compounds used were indicated earlier[2]. The melting and softening point, micro-analysis, infra-red analysis and, in addition, equilibrium surface pressure[3] of the film spread from solid particles were used as criteria of purity. Monolayers were formed from solutions in A.R. distilled (60–80° C.) petroleum ether on a clean surface of double-distilled water in an all-glass Langmuir trough. The film was compressed by means of a paraffin barrier and the pressure was measured with a horizontal film balance.

From a study of the pressure/area isotherms, it has been observed that, initially, after a region of very low pressure (~ 1 dyne/cm.) the film is transformed into a so-called liquid condensed state. On further compression, the pressure rises steeply due to the formation of a highly non-compressible, compact film in the solid state. The transition pressures for liquid condensed to solid state vary from 10–25 dynes/cm. in the case of alcohols and 25–35 dynes/cm. in the case of glycol ethers when the chain length is increased from C_{16} to C_{22}. The calculated compressibility data $k = -\left(\frac{1}{A}\frac{dA}{d\pi}\right)$ are given in Table 1.

Table 1. Compressibility (25° C.)

n-Alcohol	Liquid condensed state $k \times 10^3$	Solid state $k \times 10^4$	Glycolmono-alkyl ether	Liquid condensed state $k \times 10^3$	Solid state $k \times 10^4$
C_{16}	5·43	13·22	C_{16}	7·86	12·82
C_{18}	5·03	6·80	C_{18}	7·29	7·82
C_{20}	4·59	7·09	C_{20}	—	—
C_{22}	1·93	6·97	C_{22}	5·02	6·98

The results for the C_{16} and C_{18} *n*-alcohols are in agreement with the reported data[4]. It is noticed that the liquid condensed state is more compressible than the solid state. However, the films formed by the glycol ethers are significantly more compressible than those of the alcohols in the liquid-condensed state, which may be attributed to differences in the dipole-repulsion property and the geometry of the polar end-groups in the two series.

Following the method developed by Langmuir *et al.*[5] the specific resistance (ν) of the film was evaluated from the expression:

$$\nu = \left[A(w-w_0)\frac{t}{M}\right]_{\text{film}} - \left[A(w-w_0)\frac{t}{M}\right]_{\text{no film}}$$

where A is the area of the desiccant (lithium chloride) exposed; w and w_0, the concentrations of water vapour in equilibrium with water and desiccant respectively; M, the mass of water absorbed by the desiccant in time t sec. The temperature of the desiccant as well as of the water surface were measured by using thermistor probes through a d.c. bridge circuit. All the precautions prescribed by La Mer *et al.*[5] were taken. The results obtained at 25° C. at different surface pressures are given in Fig. 1.

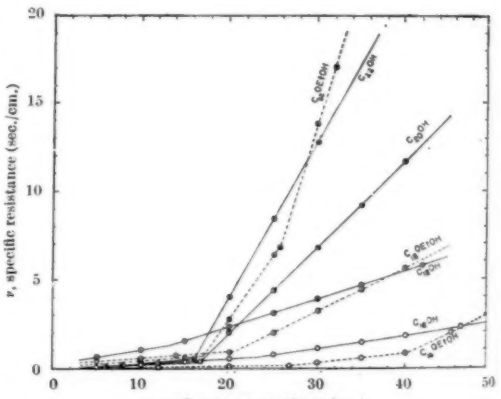

Fig. 1

The results for C_{16} and C_{18} *n*-alcohols are in agreement with reported data[6,7]. It is seen that, at lower pressures up to 16–18 dynes/cm., the resistance in all cases is very low (~1 sec./cm.). However, ν increases rapidly at higher pressures and also with the increase in the length of the chain. In the expanded condition of the film in the low-pressure region, the interaction between film molecules is very weak, requiring little energy to create a hole for the water molecule to escape. However, strong interaction between them is indicated by the sharp rise of pressure with further decrease in area when the

formation of continuous, close-packed film starts through vertical orientation of, and strong intermolecular cohesive binding between, the long chains. The observed increase of ν with the length of the chain in both the series is due to an increase in the cohesive binding between the film molecules, and hence the increase in the activation energy required to create a hole in the film.

It is interesting to note that, between the two series, the ν-values in general are lower for the glycol ethers (shown as R–OEtOH in Fig. 1) than for the corresponding alcohols at the same pressure, and this is in accordance with the fact that compressible films are less effective. After about 30–40 dynes/cm. pressure, however, the C_{22}- and C_{18}-glycol ethers exhibit higher ν-values than the alcohols and this fact supports our observations reported earlier[2], that the glycol ethers are better retarders of evaporation than the alcohols at high (equilibrium) film pressure conditions.

That the superior performance of the glycol ethers compared with the corresponding alcohols may be ascribed to higher rate of spreading (surface solution of the ethers being greatly facilitated due to the greater interaction of the polar end with the water sub-phase), higher equilibrium film pressure in the significant temperature-range, and greater adhesive interaction with the water sub-phase, will be dealt with in a separate communication.

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Electronic Properties of Imidazole

The existence of semiconduction has been demonstrated in a variety of different classes of organic compounds such as condensed aromatic hydrocarbons, charge-transfer complexes, pyrolized polymers, and stable free radicals. With few exceptions, the electrical conductivities are small, and the energies of activation for the conduction process are high. But the fact that even small conductivities are displayed suggests that some kind of intermolecular interaction takes place which, in an electric field, gives rise to electronic conduction. It is suggested that this interaction is molecular orbital overlap. The extent of orbital overlap is a function of molecular structure and hence, if semiconduction arises from such overlap, it is not surprising that semiconduction activation energies correlate with properties which are also a function of molecular structure, such as the molecular ionization energy, electron affinity, and excitation energy of the ground-triplet state transition[1,2]. One of the entities which influences these properties is the number of (the more easily polarizable) π-electrons. As the number of these increases in a conjugated system, there is a trend toward smaller activation energies of conduction, indicating that the probability of achieving overlap is enhanced by the presence of a large number of π-electrons. However, if the overlapping of molecular orbitals gives rise to semiconduction, then any other factors which contribute to such overlapping should also encourage semiconduction, even if only few π-electrons are present in the individual molecule. Since intermolecular hydrogen bonds may provide such overlap, it might be expected that activation energies in hydrogen-bonded compounds should be lower than anticipated solely on the basis of the number of π-electrons.

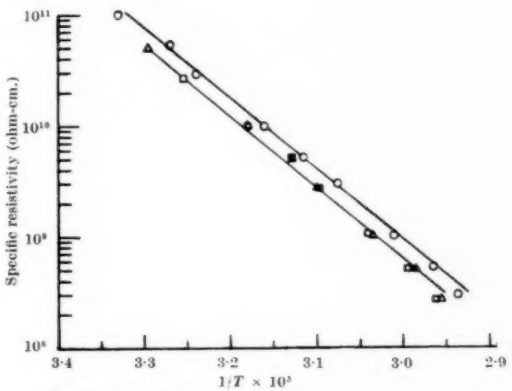

Fig. 1. Specific resistivity of imidazole. ○, run 2, 720 V./cm.; △, run 3, 72 V./cm.; □, run 4, 72 V./cm.

Supporting evidence for this view has now been found in a study of the resistivity of imidazole. Imidazole is extensively hydrogen-bonded as adduced from its relatively high melting and boiling points and from its spectra. In the present work, imidazole was purified by several recrystallizations, distillation at reduced pressure, and zone-refining. The resistivity was measured as a function of temperature on a compacted polycrystalline sample with the results shown in Fig. 1. The energy gap computed from the equation $\rho = \rho_0 \exp(E/2kT)$ was 2·6 eV. in the temperature range of 28–66°. Slightly higher resistivities were obtained at a higher potential stress; it is not known whether this is a significant effect. Energy gaps ranging between 2·8 and 3·0 eV. were obtained on several other samples which had not been as rigorously purified.

Energy gaps of compounds which contain only four π-electrons are not available for comparison with that of imidazole (which also contains four π-electrons). The energy gap of benzene (which contains six π-electrons) has not been experimentally determined but has been estimated[3] as approaching 5 eV. If the number of conjugated π-electrons were the sole factor which determines the energy gap, one might have expected that of imidazole to be much greater than the experimentally found value of 2·6 eV. It is concluded that the orbital overlap provided by hydrogen-bonding effectively facilitates semiconduction.

We thank Messrs. E. J. McGowan and J. Howard for valuable technical assistance. This research was

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Formation of Boron Nitride in Diborane-Hydrazine Flames

Although reactions of metals with nitrogen or nitrogen compounds leading to nitrides are well known[1], the literature on combustion is devoid of descriptions of flames from the reaction between boron hydrides and nitrogen hydrides (ammonia, hydrazine). This communication reports a steady-state flame of diborane and hydrazine, in which the reaction products consist principally of boron nitride and hydrogen, according to the reaction $B_2H_6 + N_2H_4 \rightarrow 2BN + 5H_2$.

Even though the value of the heat of formation of boron nitride is not fully established (27–32 kcal./mol. in ref. 2, 60·2 kcal./mol. in ref. 3) the above reaction is sufficiently exothermic for it to proceed without difficulty. Examination of this system is complicated, however, by a rapid gas-phase reaction of boron hydrides with ammonia, hydrazine or amines, leading to a variety of solid 'adducts'[4], which prevents premixing of the components prior to combustion. To overcome this problem a novel burner was constructed, consisting of a closely spaced array of capillaries (Fig. 1) by means of which a 'multi-diffusion' flame is established in a laminar mixing zone. This type of burner is of general utility for combustion studies in which premixing of components is impractical or impossible, but where it is desirable to investigate the reaction products from flames approaching the premixed state.

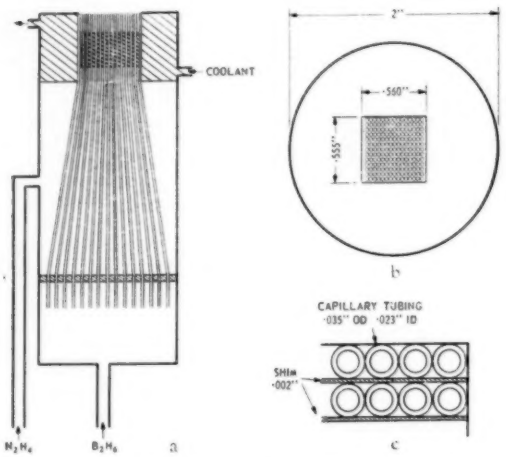

Fig. 1. Schematic diagram of capillary multidiffusion burner. *a*, Cross-section, showing arrangement of separate feeds for two incompatible gaseous components; *b*, top view; *c*, details of capillary assembly. Symmetrical assembly of tubes is essential to provide uniform gas flow at top of the burner. Metal shims are used as spacers between rows of capillaries

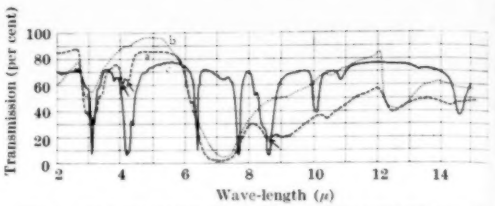

Fig. 2. Infra-red spectra of: *a*, solid reaction products from the B_2H_6—N_2H_4 flame; *b*, boron nitride; *c*, diborane-hydrazine adduct (Callery Chemical Co.). The arrowed absorption bands in *a* are due to the presence of small amounts of adduct

To achieve the boron nitride reaction a flat hydrazine decomposition flame is established first (requiring thermostatting the burner and fuel-feed above 100° C. to obtain an adequately high hydrazine vapour pressure). Subsequent addition of diborane through the separate feed system leads to a flame with copious evolution of solid reaction products consisting predominantly of boron nitride. As shown by infra-red (Fig. 2) and chemical analyses only small amounts of bound hydrogen appear as impurity in the solid.

By measurement of the flame temperature and the composition of the products of combustion an independent value for the heat of formation of boron nitride is being obtained.

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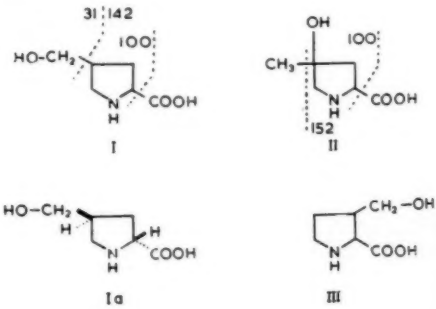
Determination by Mass Spectrometry of the Structure of Proline Derivatives from Apples

Hulme[1] and Urbach[2] independently isolated a new amino-acid from apples, and the isomeric structures I[2] and II[1,3] were proposed for these substances on the basis of elemental analysis, colour reactions and chromatographic properties[1–3]. The infra-red spectra of these compounds proved to be superimposable, and this indicated that the compounds were identical, but it did not permit a decision between the alternative structures[3].



It seemed that this problem could now be resolved by mass spectrometry, a method recently introduced into amino-acid chemistry[4,5]. Therefore, small samples of the original isolates were procured from Dr. A. C. Hulme and Mrs. G. Urbach, respectively. For these samples we are grateful.

The ethyl esters of the two compounds were prepared by treatment of 1 mgm. of each substance with boiling ethanolic hydrochloric acid and by conversion of the product into the free ester[5]. The mass spectra of the ethyl ester of the two compounds (Fig. 1) were identical in every respect: this corroborated the presumed identity[3] of the two amino-acids which had been isolated independently.

The mass spectra exhibit a very intense peak at *m/e* 100 due to loss of the carbethoxy group (73 mass units) from the molecule, the molecular weight of which is 173. This evidence relates to the structure I, and this conclusion is also supported by a peak at mass 174, which exhibits the characteristics of an $M + 1$ peak[4]. An appreciable peak is also found at mass 31 (CH_2OH) and at mass 142 ($M - 31$), which can be reconciled only with the structure I. By contrast, one would expect from structure II a considerable peak at mass 158 ($M - 15$), but none at 31 and 142, because the loss of the methyl group would lead to a carbonium ion which would, in turn, be well stabilized by the high degree of substitution as well as the oxygen atom.

Furthermore, it is now possible to deduce the stereochemistry of this amino-acid, because the mass spectrum of the ester remains substantially unchanged after the sample is kept for 30 min. in the hot (140° C.) inlet system of the mass spectrometer. A substance with the *cis*-configuration of the carbethoxy- and hydroxy-methyl groups should, under these conditions, undergo lactone formation at least in part: because this does not occur, the *trans*-structure I*a* is assigned to this compound. By the same argument, the otherwise possible structure III is eliminated, because such a substance in the form of its ester should lactonize readily under the conditions specified, whether the configuration is *cis* or *trans*.

The assignment of the optical configuration is, of course, not possible from these experiments, and this must await the final correlation of this amino-acid with proline (presumably L-proline).

After the work described, one of us (F. C. S.) learned through correspondence (letter of January 5, 1961) that Dr. G. W. Kenner and Dr. L. F. Burroughs had examined a sample identified with that of Hulme[1] and concluded that "this turns out to be a hydroxymethyl compound rather than a methylhydroxy derivative of proline, and we hope to correlate it with methyl proline". Therefore, the identity of these compounds as the *trans* form of 4-(hydroxymethyl)-proline is now established.

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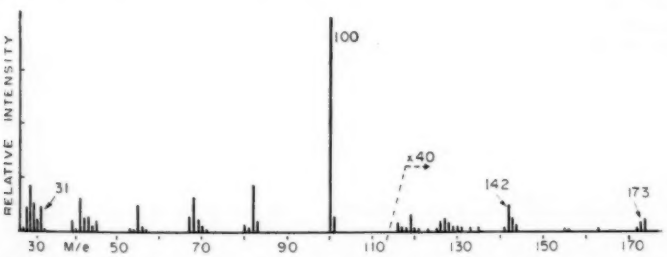

Fig. 1. Mass spectrum of the ethyl ester of the proline derivative isolated by Hulme from apples. Peaks above mass 115 are enlarged 40-fold. All peaks of ⩾ 1 per cent of the intensity at *m/e* 100 are shown below mass 116 and of ⩾ 0·05 per cent above mass 116. The spectrum of the compound isolated by Urbach was indistinguishable from that shown

Determination of Lead in Urine by Atomic Absorption Spectroscopy

Most methods for the determination of traces (0·01–1 p.p.m.) of lead in urine require prior removal of organic matter and concentration of the lead by ashing. These methods have several drawbacks: the time required for ashing; possible interference from other metals such as bismuth; and the need for careful purification of a variety of reagents to keep the blank reading at a reasonably low level.

Several methods have been described in which the lead is co-precipitated with calcium oxalate and estimated colorimetrically[1] or polarographically[2]. However, it is doubtful whether much time is saved thereby, and there is evidence[3] that under these conditions lead is not completely precipitated from urine, especially where the patient has been treated with complexing agents such as ethylenediaminetetraacetic acid or 2,3-dimercaptopropanol.

Allan[4] has recently shown that trace quantities of copper in soils and plant ash can be determined by extraction with a chelating agent into an organic solvent and spraying this solution directly into the burner of an atomic absorption spectrophotometer.

I have found that lead can be quantitatively extracted from urine by ammonium pyrrolidine dithiocarbamate[5] and methyl *n*-amyl ketone at a *p*H sufficiently low (1·5–4·5) to avoid precipitation of the phosphates. The lead may thus be concentrated by a factor of 100 or more and determined by spraying the ketone solution into the 10-cm. air-coal gas flame of the atomic absorption spectrophotometer described by Box and Walsh[6]. The absorption of the lead line at 2833 Å. is measured and the instrument is calibrated by extracting and measuring a similar quantity of urine to which a known amount of lead has been added.

Since we are concerned only with the concentration of lead in the ketone phase the partial emulsification which invariably occurs does not affect the accuracy of the determination. After shaking with ammonium pyrrolidine dithiocarbamate and methyl *n*-amyl ketone the urine is allowed to stand for 5 min., and most of the lower layer is discarded, the last 5 ml. or so being transferred, together with the upper layer and the emulsion at the interface, to a centrifuge tube. After centrifuging for 5 min. a partial separation of the layers occurs; the upper layer is removed with a fine Pasteur pipette and the remainder of the liquid stirred thoroughly and centrifuged again. The upper layer is pipetted off once more, and the combined quantities are sufficient for a measurement.

Table 1 shows the recovery of added lead from normal urine, while Table 2 compares the results obtained for pathological specimens by the dithizone method and the atomic absorption technique. It will be seen that the results of the new method are in satisfactory agreement with those of the classical one.

Table 1. Recovery of Lead added to Normal Urine (p.p.m.)

Added	Recovered
0·10	0·10
0·20	0·21
0·30	0·27
0·40	0·41
0·50	0·56

50 ml. of urine (*p*H 4) containing the added lead was extracted with 0·5 ml. of 4 per cent ammonium pyrrolidine dithiocarbamate and 1·5 ml. methyl *n*-amyl ketone. Similar specimens to which 15 μgm. of lead had been added were extracted in the same way to serve as standards.

Table 2. Comparison of Colorimetric and Atomic Absorption Methods for Determination of Lead in Urine

Specimen No.	Lead content (p.p.m.) Colorimetric	Atomic absorption
1	0·03	0·04
2	0·04	0·07
3	0·06	0·07
4	0·07	0·07
5	0·18	0·14
6	0·26	0·28
7*	0·76	0·72

* From a patient undergoing treatment with ethylenediaminetetraacetic acid.

The atomic absorption method is particularly well suited for the measurement of very low lead concentrations (<0·1 p.p.m.) as it is specific for lead and yields a negligible blank value. By using larger volumes of urine (100–200 ml.) it is possible to measure a lead concentration of 0·02 p.p.m. with a standard deviation of 0·002 p.p.m.

The method is also applicable to other heavy metals and indeed zinc and cadmium, for which the detection limit in aqueous solution is about 0·03 p.p.m.[7], can be determined by direct measurement on the urine itself without prior extraction.

I am indebted to Dr. Alan Bell and Mr. Graham Blanks for supplying the pathological specimens and for measuring their lead contents colorimetrically.

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BIOCHEMISTRY

Electrophoretic and Immuno-electrophoretic Patterns of Extracellular Diphtheria Proteins from Culture Filtrates of Different Iron Concentrations

In cultures of *Corynebacterium diphtheriae* the toxin is produced only within a relatively narrow range of iron concentration. Excess of iron inhibits toxin formation, but the mechanism of this phenomenon is not explained. Toxigenicity is strictly associated with the presence of prophage in diphtheria bacillus cells, and it has been recently suggested that iron probably affects toxin production by causing induction of the prophage–host system[1]. At iron excess a certain amount of atoxic, extracellular protein is found in the culture filtrate, but their physico-chemical and immunological properties are little known. A closer characterization of these components, which will be hereafter referred to as 'Fe-proteins', appears useful for work on toxin formation.

Paper electrophoresis has shown extracellular diphtheria proteins to constitute a complex system, comprising at least six fractions, for which the following nomenclature has been proposed[2]: fraction *D*, amounting to about 65–80 per cent, more rapid fractions ρ_1 and ρ_2, and three fractions slower than *D*, namely: σ_1, σ_2, and σ_3 (Fig. 1). Diphtheria toxin, that is, the factor endowed with lethal and dermonecrotic properties, is localized in the cathodic part of fraction *D*.

The electrophoretic pattern of 'Fe-proteins' has shown striking difference as compared with the pattern of extracellular proteins obtained at iron concentration optimal for toxin production (Fig. 2). It is characterized by a strongly diminished content

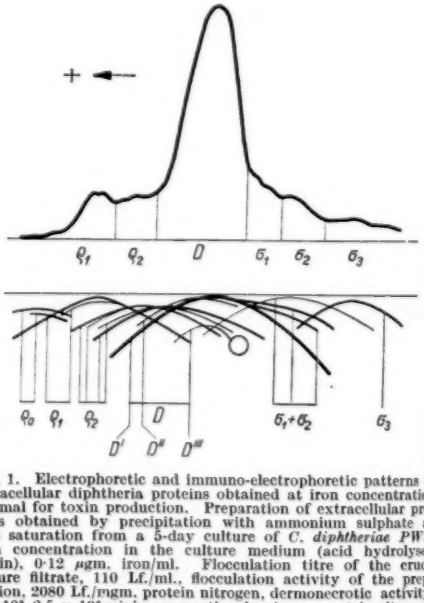

Fig. 1. Electrophoretic and immuno-electrophoretic patterns of extracellular diphtheria proteins obtained at iron concentration optimal for toxin production. Preparation of extracellular proteins obtained by precipitation with ammonium sulphate at 0·66 saturation from a 5-day culture of *C. diphtheriae PW8*. Iron concentration in the culture medium (acid hydrolysed casein), 0·12 μgm. iron/ml. Flocculation titre of the crude culture filtrate, 110 Lf./ml., flocculation activity of the preparation, 2080 Lf./mgm. protein nitrogen, dermonecrotic activity, 2×10^2–$2{\cdot}5 \times 10^2$ minimum reacting dose/mgm. protein nitrogen. Paper electrophoresis: Michaelis buffer *p*H 9·0; ionic strength, $\mu = 0{\cdot}1$. Densitometric curve after amido-black staining (ref. 2). Immunoelectrophoresis: Michaelis buffer, *p*H 9·0; ionic strength, $\mu = 0{\cdot}05$ (ref. 3)

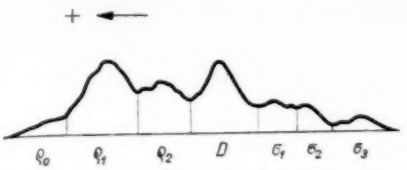

Fig. 2. Electrophoretic pattern of 'Fe-proteins'. Preparation of 'Fe-proteins' obtained by precipitation with ammonium sulphate at 0·66 saturation from a 5-day culture of *C. diphtheriae PW8*. Iron concentration in the culture medium, 1·0 μgm iron/ml. Flocculation titre of the crude culture filtrate less than 2·5 Lf./ml., dermonecrotic activity, $2{\cdot}5 \times 10^4$ minimum reacting dose/mgm. protein nitrogen. Paper electrophoresis: Michaelis buffer, *p*H 9·0; ionic strength, $\mu = 0{\cdot}1$. Densitometric curve after amido-black staining

of fraction *D*. In experiments involving determination of relative electrophoretic mobilities, it has been found that fraction *D* of the 'Fe-proteins' migrates with a mobility corresponding to the material of the anodic part of fraction *D* of extracellular proteins obtained at iron concentration optimal for toxin production. Running electrophoresis simultaneously on the same strip proved all the other fractions of the 'Fe-proteins' to be identical, with respect to their mobility, with corresponding fractions of extracellular diphtheria proteins obtained at iron concentration optimal for toxin production. In immuno-electrophoretic experiments the immunological identity of precipitating components of those fractions has been demonstrated.

The immuno-electrophoretic pattern of 'Fe-proteins' does not contain the *D′* line, which is the principal component of the pattern of extracellular diphtheria proteins obtained at optimal iron concentration[3]. When, however, immuno-electrophoretic patterns of extracellular proteins obtained at iron concentration optimal for toxin production were developed with anti-toxic sera absorbed with 'Fe-proteins' they were found to contain two additional precipitation lines besides line *D′*: line *D‴* and one localized within fraction ϱ_2 (Fig. 3). These experiments have clearly demonstrated that excess iron inhibits the production of at least three precipitating components.

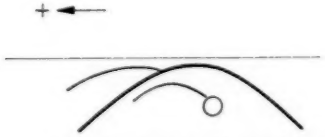

Fig. 3. Immuno-electrophoretic pattern of extracellular diphtheria proteins obtained at iron concentration optimal for toxin production. Flocculation activity of the preparation used, 1,900 Lf./mgm. protein nitrogen. The pattern was developed with anti-toxin absorbed with 'Fe-proteins'

It may be concluded that the culture filtrate of *C. diphtheriae PW8* contains macromolecular components which differ in the mechanism of origin. The components of fractions ϱ and σ are produced at iron concentration optimal for toxin formation and at a concentration which almost completely inhibits toxin production. Fraction *D* appears to be characteristic of culture filtrates of toxigenic strains of *C. diphtheriae* at iron concentration favourable for toxin production. The effect of iron, however, is a complex one, because the inhibition of production of at least three precipitating components, one of which may be identical with the toxin, is simultaneously observed.

The toxic activity of extracellular diphtheria proteins is localized within fraction *D*. However, the asymmetric distribution of the activity within this fraction, as well as the results obtained on electrophoresis in buffers of low ionic strength, clearly demonstrated its heterogeneity[2]. Fraction *D* contains also considerable amounts of glycoproteins, which may be only partially separated as the more mobile borate-separable fraction D_1 on electrophoresis in borate buffers[2]. It seems that an explanation of the part played by different macromolecular components present in the culture filtrate should throw some light on the mechanism of toxin formation.

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Formation of 5-Hydroxytryptamine in an Aqueous Solution of 5-Hydroxytryptophan treated with X-rays

IT has been found that 5-HT (5-hydroxytryptamine) is formed by the action of ultra-violet irradiation on 5-HTP (5-hydroxytryptophan) aqueous solution, as a result of a decarboxylation process[1]. It is of interest to investigate whether the same process takes place under the influence of X-irradiation.

An aqueous 0·1 per cent solution of 5-HTP as a pure base, contained in a 'Pyrex' glass vessel, was irradiated with X-rays generated from a Philips machine, operating at 200 kV. and 20 m.amp. (The glass vessel was in close contact with the target.) The dose-rate was 1,818 r./min., measured by the ionization chamber. The solution was prepared in re-distilled water and exposed to X-irradiation for 10 hr. The thickness of the solution layer was 5 mm. The loss of water caused by evaporation during the exposure time was compensated every 2 hr. The irradiated solution was tested for 5-HT on atropinized œstrous rat uterus[2].

After the irradiation of the solution a brown coloration was noticed. This brown solution showed an oxytocic activity. In preliminary experiments oxytocic activity of the starting material (5-HTP) was examined and we found that 5-HTP *per se* possesses a slight activity on rat uterus, the sensitivity threshold being 0·2 mgm./ml. We were obliged, therefore, to work with the starting solution diluted below this threshold concentration of 5-HTP. After eliminating the interference with 5-HTP, we found that the solution still showed oxytocic activity. The inhibition experiments with lysergic acid diethylamide—a specific antagonist of 5-HT—showed that this activity is to be ascribed to 5-HT. The concentration of 5-HT in the irradiated solution was found to be rather low (0·004 per cent), the *G*-value being 0·2.

Preliminary analysis of descending paper chromatography of the irradiated solution (solvent system: butanol–acetic acid–water (4 : 1 : 5); Ehrlich's reagent) showed, besides 5-HTP ($R_F = 0{\cdot}30$), four more spots with the following R_F values: 0·12; 0·36; 0·46 and 0·75. The spot with the R_F value of 0·46 corresponded to 5-HT in location, but not in colour.

It has been demonstrated that, in X-irradiated aqueous solutions of histidine[2] and probably tyrosine[4], decarboxylation occurs to a very low amount. This is possible, because in aromatic amino-acids there are a number of sites to be attacked by the free radicals produced from the water. Our results with rather small G-value indicate that 5-HTP (an aromatic amino-acid too) behaves similarly.

It has been noted[5] that 5-HT in aqueous solution undergoes many changes in its structure under the influence of different oxidizing agents. Taking into account the low conversion-rate of 5-HTP to 5-HT, one should consider the possibility of transformations of 5-HT molecules when under the influence of X-irradiation. It seems to us likely that the low amount of 5-HT formed could be partly explained by the action of the various oxidizing species produced by the X-rays.

We wish to thank Dr. A. J. Swallow, London, for his interest and helpful suggestions. 5-HTP was kindly supplied by the National Drug Company, Philadelphia, Pennsylvania.

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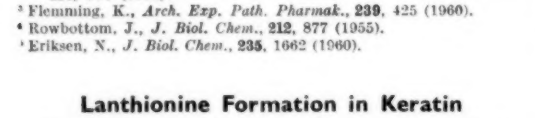
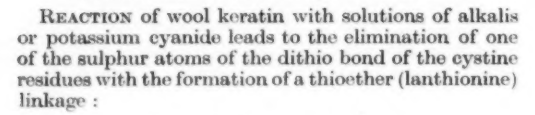
Lanthionine Formation in Keratin

Reaction of wool keratin with solutions of alkalis or potassium cyanide leads to the elimination of one of the sulphur atoms of the dithio bond of the cystine residues with the formation of a thioether (lanthionine) linkage :

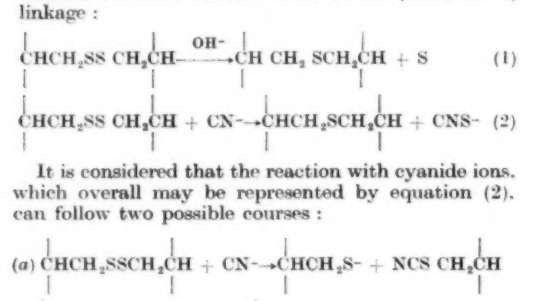


(1)

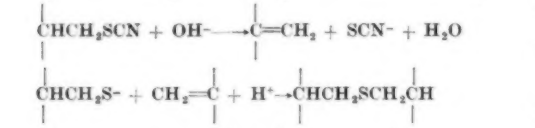


(2)

It is considered that the reaction with cyanide ions, which overall may be represented by equation (2), can follow two possible courses :

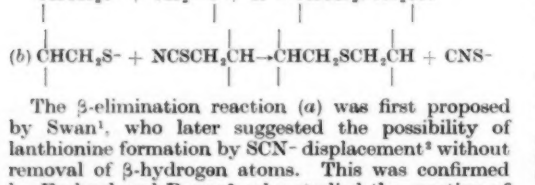


The β-elimination reaction (*a*) was first proposed by Swan[1], who later suggested the possibility of lanthionine formation by SCN^- displacement[2] without removal of β-hydrogen atoms. This was confirmed by Earland and Raven[3], who studied the reaction of N-mercaptomethylpolyhexamethyleneadipamide disulphide with cyanide ions, and showed that a thioether bond is formed under conditions identical to those which produce lanthionine in wool :



Since β-elimination is impossible, it must be concluded that a reaction such as (*b*) is very probably an important route for lanthionine formation in wool.

On the other hand, in the alkaline reaction, Swan[4] has shown the importance of a β-elimination mechanism :



since αα′dimethylcystine is degraded only extremely slowly under these conditions, compared with free cystine.

The reaction of N-mercaptomethylpolyhexamethyleneadipamide with alkalis has now been examined, as it is probably a better model for wool[6] than Swan's compounds, in that the reaction, as with wool, is heterogeneous, giving the ruptured disulphide residues a greater chance to re-unite in a lanthionine linkage.

2·00 gm. of disulphide (sulphur, 4·20 per cent) were boiled under reflux for 5 hr. with 400 ml. of the solution of alkali. After reaction, the solid product was collected, washed with water and dried *in vacuo*. The results obtained are given in Table 1. The analytical methods used have been described previously[3].

Table 1

Solution	pH	Wt. loss (per cent)	Sulphur in product (per cent)	Disulphide sulphur (per cent)	NH_2 m.equiv. 100 gm.
—	—	—	4·20	4·07	1·5
0·10 *M* Na_2HPO_4	8·0	1·0	3·99	4·26	1·5
0·05 *M* $Na_2B_4O_7$	9·2	0	4·14	4·27	1·6
0·10 *M* Na_2CO_3	10·5	2·5	3·70	3·94	2·3
0·10 *M* Na_2CO_3*	10·5	11·0	3·39	3·28	2·7
0·10 *M* NaOH	12·0	35·0	2·23	1·90	4·7

* Boiled for 10 hr.

It is shown that a lanthionine type of reaction does not occur when the β $\diagdown\!\!\diagup$CH group is replaced by nitrogen, which must be regarded as strong supporting evidence for the β-elimination mechanism, and against mechanisms which involve formation by direct attack on the α-carbon atom[5].

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Effect of Polyamino Carboxylic Acids on Catalase

CATALASES are known to be inactivated by the formation of compounds with cyanide[1], azide and hydroxylamine[2], so we thought it probable that the iron of the enzyme could be complexed with chelating agents, the effect being also to inactivate the enzyme. The chelating agents used were ethylenediamine tetraacetic acid (EDTA), cyclohexanediamine tetraacetic acid (CDTA), diethylenetriamine pentaacetic acid (DTPA) and NN′ethylene*bis*(2{*o*-hydroxyphenyl}) glycine (EHPG). The rate of decomposition of hydrogen peroxide by bovine-liver catalase in the presence of each of the above chelating agents was followed by a rapid titration method.

A decrease in the rate of destruction of hydrogen peroxide by the catalase was observed in the presence of all the chelating agents. With EDTA and DTPA, the inhibition reaches a maximum at a *p*H of about 8·5 and then decreases again with increasing *p*H. With CDTA, the inhibition increases slowly with increasing *p*H, whereas with EHPG the increase is rapid, although measurements could not be made at *p*H values greater than 10 due to a rapid decrease in the activity of the catalase, probably due to protein denaturation. In conclusion, it must be noted that the inhibition of catalase by these chelating agents is small compared to that of hydrogen cyanide, which has a value of K_i of $4{\cdot}6 \times 10^{-6}$ *M* at *p*H 7 (ref. 3), whereas at *p*H 8·5 (*p*H of maximum inhibition of catalase in the presence of EDTA and DTPA) the K_i values are $9{\cdot}2 \times 10^{-2}$ *M*, $9{\cdot}3 \times 10^{-2}$ *M*, $2{\cdot}6 \times 10^{-1}$ *M*, and $3{\cdot}3 \times 10^{-1}$ *M* for EDTA, DTPA, CDTA and EHPG respectively.

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Dextransucrase V and the Role of Metal Ions in Enzyme Catalysis

DEXTRANSUCRASE is a representative member of the general class of enzymes known as transglycosidases. In this particular example, the glucosyl group from sucrose is transferred to a suitable acceptor, which causes chain initiation, and dextran is formed. Polymer-formation is governed by many factors such as the nature of the acceptor molecule, *p*H and temperature[1].

This work was directed at the role of metal ions in enzyme catalysis[2]. This was initiated by some previous work which had suggested that magnesium plays a part in dextran synthesis[3,4]. These investigations had shown that a relatively linear molecule of dextran could be isolated from a magnesium-deficient diet inoculated with *Betacoccus arabinosaceous* (Birmingham). This is contrasted with the branched dextran isolated from a normal medium. With this in mind, it was felt important to study the effect of metal ions on the enzyme and to carry out a detailed kinetic investigation.

Using fructose-production as a measure of enzyme activity, the conversion of sucrose to dextran was examined under the influence of several metal cations. The details of the enzyme preparation and the analytical procedures have been reported[2]. In addition to testing the effect of the metal ions on the catalysis, the role of metal chelating agents, tetrasodium ethylenediamine tetraacetic acid (EDTA) and oxalic acid were investigated. All experiments were conducted in a 0·05 *M* acetate buffer of *p*H 5·2 and the reaction was carried out for 0·5 hr. in a constant temperature water bath at 25° C.

Of the metal ions tried as activators of this particular enzyme, notably calcium, zinc, magnesium, manganese and copper, only the first was effective. The reciprocal plot of initial velocity against calcium ion at various substrate concentrations is shown in Fig. 1. From the common intercept of these lines, a value for the dissociation constant of the enzyme metal complex may be evaluated. This turns out to be $9{\cdot}4 \times 10^{-5}$ *M*. The action of EDTA is shown in Fig. 2. The graph for oxalic acid was similar, except

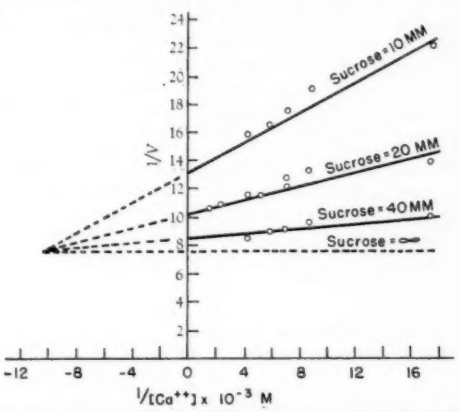

Fig. 1. Reciprocal plot of initial velocity (mgm. fructose/0·2 ml. enzyme/0·5 hr.) against calcium ion concentration. The dashed curve representing sucrose = ∞ was drawn through the intersection of the three other curves and the value for $1/V$ calculated from another experiment using enzyme and sucrose and an excess of calcium ion

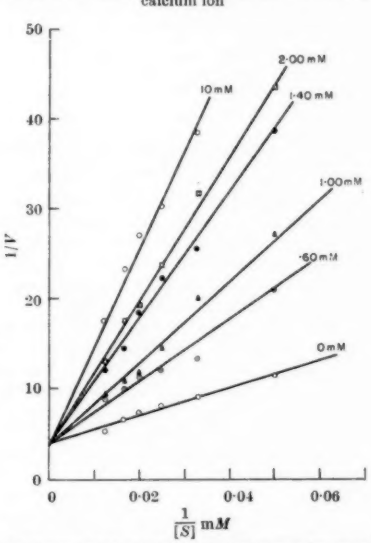

Fig. 2. Reciprocal plot of initial velocity (mgm. fructose/0·2 ml. enzyme/0·5 hr.) against sucrose concentration at various levels of EDTA

that it required twenty-five times as much oxalic acid to achieve the same results.

The plot in Fig. 1 showing the influence of calcium ions fits the first type of coupling activation as described in ref. 5. In this particular case of activation, the substrate will not couple efficiently with the enzyme unless the activator (in this case calcium ion) is already attached to the enzyme. Competitive inhibition is demonstrated in Fig. 2 for the action of the chelating agents. The action of oxalic acid and EDTA appears to be a removal of the calcium ion from the enzyme. This removal renders the catalytic site less active in its action on sucrose. In the presence of excess substrate the influence of the chelating agents is diminished by the law of mass action, as demonstrated by the convergence of the lines in Fig. 2 to a common intercept.

The ability of magnesium ion to influence the degree of branching has been mentioned[3,4]. In view of this fact, it is rather surprising at first sight that magnesium appeared to have no influence on the course of the reaction in this work. A possible explanation for this apparent anomaly might rest on the following argument: according to Bovey[6,7] there are at least two enzymes responsible for the synthesis of dextran. One enzyme operates by initiating chain-growth and liberating fructose. The other, the so-called 'branching enzyme', operates by transferring pre-formed units of oligosaccharides to the main chain. This latter enzyme would not liberate any reducing sugars; consequently it would not be detected by the methods of analysis used in this investigation.

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Degree of Polymerization of Hyaluronic Acid isolated from Human Aortic Tissue

Several reports have been presented on the degree of polymerization of hyaluronic acid isolated from umbilical cords, ovarian cysts and follicular fluid, synovial fluid and vitreous bodies[1–5]. The hyaluronic acid isolated from the tissues mentioned shows varying degree of polymerization.

In a previous publication Bertelsen and Marcker[6] discussed variations in the ratio chondroitin sulphuric acid/hyaluronic acid in aortic tissue with age. In this investigation the degree of polymerization of hyaluronic acid isolated from human aortæ has been determined by means of osmometry.

The aortæ were obtained 12–15 hr. after death. The adventitia was carefully removed by hand, and the media-intima parts of the vessels were stored in acetone for 4–5 months. Owing to the small amount of tissue in children, samples from individuals less than 2 yr. of age were pooled. Furthermore, three groups of aortæ from the new-born were included in the material, each of the groups consisting of 10–12 aortæ. Only macroscopically normal tissue was collected, and so, if the question were about elderly individuals with atherosclerotically changed aortæ, the changed parts were discarded.

The disintegrated, de-fatted tissue was incubated with a proteolytic enzyme, and the protein-split products were precipitated with trichloroacetic acid. The residual protein-split products were removed by repeated shakings with chloroform–*iso*amylalcohol[2] and subsequent dialyses against running tap-water. A more detailed description of the method of extraction was given in ref. 6.

In order to remove the chondroitin sulphuric acids, the aqueous solution of mucopolysaccharides was brought to *p*H 1·5 with 0·1 *M* hydrochloric acid in the presence of 0·1 *M* sodium chloride at 0° C. At this *p*H the ionization of the hyaluronate is suppressed, whereas the chondroitin sulphates are largely ionized. 1 gm. of 200–400-mesh amine ion-exchange resin 'Dowex 1–*X*8' in the chloride form per 25 ml. of the mucopolysaccharide solution was added, and the mixture was stirred for 2 hr. at 0° C. (ref. 8). After removal of the resin by centrifugation and filtration through a glass funnel, the *p*H was raised, and by slowly adding two volumes of cold absolute alcohol, the hyaluronate was precipitated. The precipitate was washed in absolute alcohol and ether and dried in a vacuum desiccator containing silica-gel.

To characterize the isolated substances, we used nitrogen and sulphur determinations, viscometric determinations and subsequent incubation with streptococcal hyaluronidase and paper chromatography. The nitrogen was determined by the micro-Kjeldahl procedure, and the sulphur contents of the hyaluronic acid preparations were determined by the method of Zinneke[9]. Viscometric investigations were performed in the micro-viscometer described in ref. 10. Paper chromatography of hydrolysates of the substances was performed in 2,6-lutidine/water (65 : 35) for 24 hr., and the chromatograms were developed by a 0·1 per cent ninhydrin solution in acetone.

The following tends to show that the isolated substances really were hyaluronate: the white powders were: (1) highly hygroscopic; (2) easily soluble in water, the solutions being clear and viscid; (3) the viscosities decreased considerably after incubation with bacterial hyaluronidase, giving the characteristic degradation curves of hyaluronic acid. Furthermore, (4) the paper-chromatograms of the hydrolysates only showed spots corresponding to authentic glucosamine, and (5) the amount of nitrogen in all samples was about 3·6 per cent (theoretically 3·4). The sulphur in the samples ranged from 0·6 to 0·8 per cent (with one exception), thus indicating only minor amounts of sulphated mucopolysaccharides.

The molecular weights were calculated from colloid osmotic pressure measurements performed by the micro-osmometer described in ref. 2. The concentration used in the osmotic pressure measurements was 5 mgm. of the isolated substance in 1 ml. 0·5 *M* potassium chloride. According to our experience the influence of the Donnan effect is then negligible.

The results are shown in Table 1. It will be seen that the degree of polymerization of hyaluronic acid in aortic tissue is independent of age and degree of atherosclerosis.

The isolation procedure used in this investigation is generally considered very sparing, and it is unlikely that any significant depolymerization occurred during the isolation.

Table 1. MOLECULAR WEIGHTS (M) OF HYALURONATE ISOLATED FROM HUMAN AORTÆ, CALCULATED FROM MEASUREMENTS OF THE OSMOTIC PRESSURES

Age (yr.)	Sex	$M \times 10^3$	Nitrogen (per cent)	Sulphur (per cent)	Gross appearance
0		44	3·62	0·69	No plaques
0		43			No plaques
0		35			No plaques
0·5–2*		51	3·59		No plaques
6	♂	30	3·18	0·63	No plaques
11	♂	48		0·61	No plaques
35	♀	48	4·29		Lipoid plaques
50	♂	43	3·52	0·76	Slight sclerosis
60	♂	47	3·62		Slight sclerosis
63	♀	40		0·73	Pronounced sclerosis
72	♀	42	3·44	1·30	Pronounced sclerosis
84	♂	60	3·80	0·81	Pronounced sclerosis

* This group consists of three aortæ.
Owing to the small amount of substance at our disposal, only some of the results with nitrogen and sulphur have been determined.

The molecular weights presented in Table 1 refer to apparent molecular weights, and not to true molecular weights; the latter, which are obtained by extrapolating to zero concentration, are, therefore, a little higher than those recorded.

It can be concluded that the hyaluronic acid isolated from aortic tissue seems to have a comparatively low molecular weight as compared with the molecular weights obtained for hyaluronic acid isolated from other tissues.

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Incorporation of Deoxyribonucleic Acid by Mammalian Cells *in vitro*

TRANSFORMATION of bacterial cells in the presence of deoxyribonucleic acid (DNA) has been demonstrated[1–3], and has indicated that genetic information is carried by DNA. There is little unequivocal evidence for such transformations in higher forms of life, in contrast to the obvious transfer of genetic information that occurs by means of the gamete during fertilization. The two reported attempts to obtain these transformations through the mediation of added DNA have been conflicting[4,5]. On the other hand, evidence for the uptake of DNA by mammalian cells[6,7] has supported the idea that observed biological effects might be obtained. In this communication I present evidence for the incorporation of extracellular high molecular weight DNA into the nuclear DNA of cells of the Ehrlich–Lettré ascites tumour, when they are incubated *in vitro* (Table 1). Labelled homologous DNA for these experiments was first obtained by injecting formate labelled with carbon-14 intraperitoneally in mice bearing this tumour. After an appropriate time-interval, the tumour cells were removed and washed and the DNA isolated by the detergent procedure[8]. To determine the specific activity of the DNA used in the experiments, samples were hydrolysed with 70 per cent perchloric acid for 1 hr. at 100° C. and the bases, adenine and thymine, separated by paper chromatography using a solvent system of *iso*propanol, hydrochloric acid and water. The bases were eluted and the specific activity of each was determined for appropriately plated samples using a gas-flow counter for the radioactivity determinations.

Table 1. *In vitro* INCORPORATION OF DNA LABELLED WITH CARBON-14 BY EHRLICH-LETTRÉ ASCITES TUMOUR CELLS

	Specific Activity (counts/min./μmole base)		
	Adenine	Thymine	Ratio adenine : thymine
Initial DNA, from *in vivo* labelling	3,852	1,395	1·00 : 0·36
Nuclear DNA after incubation	296	93	1·00 : 0·31

Incubation conditions: 2 ml. ascites fluid (0·5 ml. packed cells), 1 ml. 0·25 M sucrose—0·1 M phosphate buffer pH 7·4, 0·8 ml. 0·1 M glucose in 0·25 M sucrose, 0·2 ml. water. Incubated aerobically at 37° C. for 1 hr. in a metabolic shaking incubator.

In the incubation experiments tumour cells were obtained by draining the fluid from an abdominal incision in mice bearing a tumour growth of 7–10 days. The cells were incubated aerobically in a shaking incubator at 37° C. in the presence of ascites serum, 0·25 sucrose–phosphate buffer pH 7·4, glucose[9], and with samples of the intact labelled DNA dissolved in the buffer solution. After 1 hr. the cells were washed carefully with the sucrose–phosphate buffer and saline, and were then frozen in a dry-ice/acetone mixture. The frozen cells were homogenized in dilute citric acid (0·05 M) and fractionated by differential centrifugation to obtain preparations of clean nuclei. This procedure is of importance to ensure that the observed uptake is in nuclear material. The isolated nuclei were washed several times during the preparation with 0·01 M citric acid and were extracted finally with cold 4 per cent perchloric acid and lipid solvents[10]. The dry residual nuclear material was hydrolysed with 0·3 N potassium hydroxide for 18 hr. at 37° C. to separate the nuclear ribonucleic acid (RNA). DNA was obtained by acidifying the alkaline hydrolysate and extracting the residue with 4 per cent perchloric acid at 90° C. for 15 min. The DNA fraction was dried, then hydrolysed with 70 per cent perchloric acid as before and the bases separated by paper chromatography using the solvent system described.

The amount of DNA taken up by the nuclei under these conditions can be calculated from the total number of counts added before, and obtained after, the incubation. In these experiments it has been found that there is an observed uptake of 2–4 per cent of the added DNA by the nuclei of these cells. In looking further, in an examination of the specific activity of the nuclear DNA, it has been found that despite an expected dilution the ratio between the specific activity of adenine and thymine is similar to that of the original sample of DNA used in the experiments. While it is recognized that these observations might be explained by assuming a prior breakdown of the DNA followed by its re-synthesis in the cell nucleus, this seems unlikely, because of the difference in the degree of labelling of the bases adenine and thymine of DNA, under *in vivo* and *in vitro* conditions (to be described elsewhere). It is concluded that some intact extracellular DNA of high molecular weight can be transferred across the

cell and be incorporated into the genetic fine material of these cells. Further attention is being directed to the conditions necessary for this transfer and incorporation and to the biological effects elicited by such incorporation.

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Anticancer Properties of Thio-diglycollic Acid

In earlier publications[1–4] we have shown possibilities of preferential inhibition of tumour growth by antimetabolites of hexose monophosphate (HMP) pathway. A number of derivatives of thiophene-dicarboxylic acid have been prepared and tested for their anticancer properties. Results with thiophene 2,5 dicarboxylic acid (TDA) and 2,5 dicarbethoxy-3,4 dihydroxy thiophene (DICETOL) have already been published[3,4]. Results with other compounds will be published in detail elsewhere. One of the compounds synthesized in this series was thio-diglycollic acid (TDGA). In this compound carbon numbers 3 and 4 from thiophene have been completely dispensed with. This, in a way, amounted to an attempt to identify the active groupings which may be responsible for the anticancer properties.

Thio-diglycollic acid was synthesized by treating mono-chloro acetic acid with sodium sulphide, as described by Barkenbus and Landis[5]. This compound is extremely soluble in water. It was therefore isolated from aqueous solutions by continuous liquid-liquid extractions with ether.

Table 1. Analytical Data

Calculated	C = 32·00 per cent	H = 4·00 per cent
Found	C = 31·50 per cent	H = 3·60 per cent
Melting point:	Literature, 129°;	Found, 129–131°

The anticancer property of this compound was tested against two transplantable tumours: (1) Yoshida sarcoma (ascites) in rats and (2) solid fibrosarcoma in Swiss mice[6].

Thirty-six Wistar female rats, 2·5–3 months old, were transplanted intraperitoneally, under aseptic conditions, with 0·6 ml. each of freshly withdrawn Yoshida sarcoma ascitic fluid. After 8 hr., the animals were randomly divided into two groups—control and experimental—of 18 animals each. Control animals received 0·5 ml. of isotonic saline, while the experimental animals were given 2 mgm. of TDGA dissolved in 0·5 ml. of isotonic saline intraperitoneally. The treatment was given three times a day, with a total dose of 6 mgm. of TDGA/rat/day (40 mgm./kgm. of body weight). Multiple daily injections were necessary to maintain adequate drug concentration at the tumour site throughout the day. All the rats from the control group died between the fourth and ninth day after transplantation. TDGA administration to the experimental group was discontinued after the tenth day and survival observed. Out of the eighteen animals only two animals died 35 days after transplantation with solid growth in the peritoneal cavity. The remaining sixteen rats from the experimental group have survived for more than 5 months after transplantation, even though the drug administration was discontinued on the tenth day (Fig. 1). Since Yoshida sarcoma is a very rapidly growing tumour, it was anticipated that, even if a few viable cells had been left behind, the tumour growth would have flared up and killed all the transplanted animals within 10 days after discontinuation of the treatment. But the fact that sixteen out of eighteen animals have survived for more than 5 months after the discontinuation of the treatment suggests that Yoshida sarcoma tumour cells have been completely wiped out by TDGA treatment. All the animals from the experimental group are normal and do not show any toxic side-effects. The animals are still under observation.

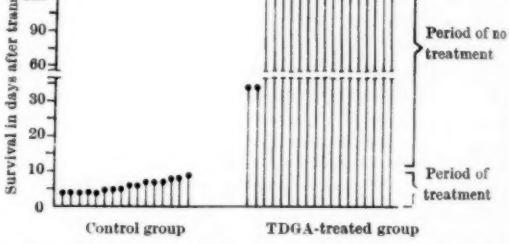

Fig. 1. Effect of TDGA on survival of Yoshida sarcoma (ascites) bearing rats. ●, Rats dead; ▲, rats living

TDGA is an acidic substance which was directly injected into the peritoneal cavity. In an auxiliary experiment, acetic acid in similar concentration was injected in the peritoneal cavity of Yoshida sarcoma-bearing rats and survival observed. It was found that the animals treated with acetic acid died more or less at the same time as the control group, suggesting that the therapeutic effects observed with TDGA were not due to acidity of the substance.

Influence of TDGA was also studied on transplantable solid fibrosarcoma in Swiss mice[6]. For this purpose, fibrosarcoma was implanted by subcutaneous injections of 0·5 ml. of tumour suspension, and the treatment started after the thirteenth day when the tumour attained measurable and uniform size in all the animals. TDGA was injected subcutaneously twice a day and the total daily dose was 6 mgm./day (240 mgm./kgm. of body weight). The animals were killed after 12 days treatment (twenty-fifth day after transplantation). The results are shown in Table 2. It will be seen that, although the results with solid fibrosarcoma were not as spectacular as with Yoshida ascites tumour, there was definite evidence of inhibition of tumour growth. The average weight of tumour from the control animals was 4·75 gm. as against 2·8 gm. in the TDGA-treated animals.

TDGA did not show any toxic side-effects with the therapeutic doses employed. Large amounts of

Table 2. EFFECT OF THIO-DIGLYCOLLIC ACID (TDGA) ON GROWTH OF FIBROSARCOMA IN SWISS MICE*

Treatment	Dose	Day of treatment after transplantation	Period of treatment (days)	Average tumour weight and range (gm.)
Treated	6 mgm./day	13th	12	2·829 (1·470–3·628)
Control	—	13th	12	4·755 (4·378–6·273)

* 6–8 weeks old Swiss female mice were used in these experiments.

TDGA, at a dose of 1,250 mgm./kgm. body-weight, were injected at one shot subcutaneously. Except for the skin necrosis seen at the site of injection, the treated animals did not show any toxic side-effects. Further work on the mechanism of action is in progress. TDGA has two acidic groups, and hence, in addition to studying its role as an antimetabolite of pentose phosphate pathway intermediates, its action as a potential bifunctional group is also under investigation.

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HISTOCHEMISTRY

Muscle Target Fibres, a Newly Recognized Sign of Denervation

THE muscle target fibre, a newly recognized sign of skeletal muscle fibre denervation, as observed in muscle biopsies from patients with neuromuscular disease, is described in this communication. From a series of forty-six biopsies showing the classic features of denervation[1] (associated with a variety of diseases), 27 specimens also contained target fibres. Eight specimens had 3–10 target fibres, four had 10–20 target fibres, and fifteen had more than twenty target fibres. The presence of target fibres could not be correlated with any particular associated disease. A concurrent series of 25 muscle biopsies showing the classic features of myopathy[1], associated with a variety of diseases, did not contain any target fibres. A concurrent series of 29 muscle biopsies having no pathological features did not contain any target fibres.

The name 'target fibre' is derived from the appearance on cross-section (Figs. 1*a*, 2*a*, *b*). Each muscle fibre so affected has three more or less distinct concentric zones, numbered 1, 2, and 3 from the centre outward, although these is some variability in this pattern. Occasionally zone 2 is absent. More than one target within a fibre is rare. On longitudinal section

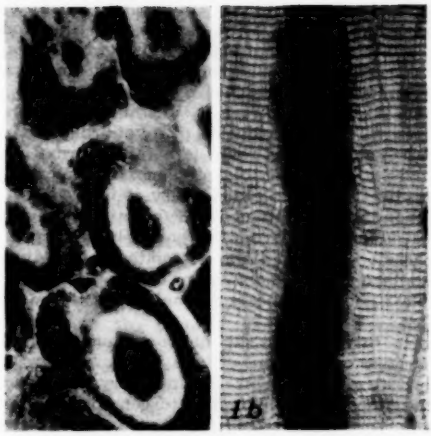

Fig. 1. *a*, Phosphotungstic acid hæmatoxylin stain (× 560); *b*, Gomori trichrome stain, polarized light. The anisotropism of zone 1 is masked by the stain (× 560)

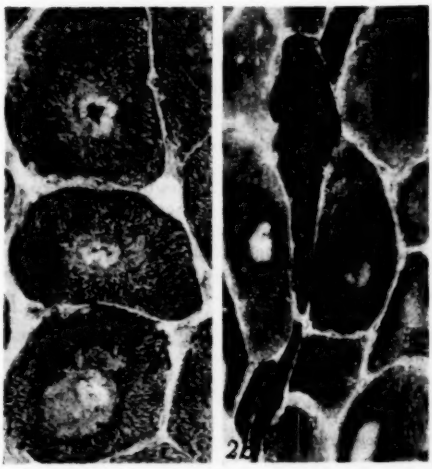

Fig. 2. *a*, Lactate dehydrogenase (× 225); *b*, alkaline adenosine triphosphatase (× 225)

through the centre of the target fibre, five parallel-bordered zones appear (from one side to the other—3, 2, 1, 2, 3), running the length of the fibre present on the slide (Fig. 1*b*). In paraffin-embedded sections, target fibres are best demonstrated after fixation in Bouin's fluid and staining by the phosphotungstic acid hæmatoxylin or the Gomori trichrome method.

Zone 1, in paraffin sections (Figs. 1*a*, *b*), contains compact wavy longitudinally running strands of myofibril-like material, which lack cross-striations and are uniformly anisotropic in polarized light. In fresh frozen sections, histochemical evidence of mitochondrial oxidative enzyme activity (succinate dehydrogenase[2], lactate dehydrogenase, cytochrome oxidase[3]) is completely absent (Fig. 2*a*). Evidence for the presence of glycogen (periodic acid–Schiff stain), for the means of glycogen formation (glucose-1-phosphate + uridine triphosphate via uridine-diphosphate-glucose to glycogen[4]), or for the means of glycogen breakdown (amylophosphorylase reaction[5]), which are sarcoplasmic reactions, is completely

absent. Alkaline adenosine triphosphatase activity[6] is absent (Fig. 2*b*). In a few instances, within zone 1 there is a small region of high activity of the oxidative enzymes (Fig. 2*a*), the glycogen metabolism enzymes, and the periodic acid–Schiff reaction.

Zone 2 appears virtually unstained in paraffin sections. The concentration of myofibrillar material appears reduced, but there is a faint normal cross-striated pattern detectable in polarized light. Alkaline adenosine triphosphatase activity is increased. The mitochondrial oxidative enzyme reactions are usually decreased or absent, but infrequently they may be increased. The reactions associated with glycogen metabolism may be increased or decreased.

Zone 3 usually appears normal by all the methods used.

In the target fibres, the nuclei are not abnormal in appearance or location. There is not an increase of connective tissue or an inflammatory cell reaction around the target fibres. The target fibres are either of about normal diameter or are somewhat smaller than normal. They may appear in a group suggesting a motor unit, but such a pattern cannot always be determined, especially when their number is small or very large.

One must distinguish target fibres from the muscle fibres containing central cores in central core disease[7–9]. The central core fibres differ from target fibres in the following features: the central core, in paraffin sections or after histochemical staining, appears as a central uniform region (or regions) within the muscle fibre. With nearly all the ordinary histological techniques, the central core is more darkly stained than the surrounding non-core region. On longitudinal section, the central core has well-preserved cross-striations. There are no classic features of denervation in the muscle of central core disease, but there infrequently may be features of an active myopathy[9].

The work described here indicates that the target fibre represents a pathological alteration of the cyto-architecture of the human skeletal muscle fibre, which has been found only in association with denervation. It is concluded that the target fibre is a sign of skeletal muscle denervation. As the target appearance occurs in fibres of normal or somewhat smaller than normal diameter, it probably signifies recent denervation.

Most of the muscle biopsies reviewed were generously provided by Dr. G. Milton Shy. Prof. W. Blackwood kindly permitted study of the specimen shown in Fig. 1*a*.

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RADIOBIOLOGY

Assessment of Chronic Exposure to Radiostrontium by Urinary Assay

Radioactive contamination of the biosphere and particularly of the human population is a contemporary problem of high interest, and strontium-90 is perhaps the most important single radionuclide concerned. We require to know what levels are being incorporated into the human population at any given time. Since food is the major route by means of which environmental radiocontaminants reach human beings, attention has largely been turned to the monitoring and surveying of food and water. This communication proposes that urinary assays of strontium-90 and calcium may have considerable advantage for the estimation of the contemporary strontium-90 to calcium ratio of the intake, and for estimation of the strontium-90 to calcium ratio in newly forming bone. The same considerations would also apply to strontium-89 whenever this radionuclide is of inportance.

Several investigations have shown that, under typical conditions, the strontium to calcium ratios between diet, various tissues and secretions are roughly constant. Examination of results obtained by independent methods in many laboratories and countries has indicated that under usual dietary conditions the values of the ratio (strontium/calcium of sample (ref. 1)) to (strontium/calcium of precursor), called here the 'observed ratio', fall within a reasonably narrow range, with few indications of variations as high as a factor of 2. Some typical values are as follows: observed ratio (body/diet) for human beings, 0·25–0·50 (refs. 2–4); observed ratio (body/diet) for cattle, sheep, pigs, goats, 0·2–0·25 (refs. 2 and 5); observed ratio (milk/diet) for human beings, cow and goat, 0·09–0·16 (refs. 2 and 4).

An example of the usefulness of this concept is the estimation of the comparative levels of strontium-90 and calcium in the total dietary intake of dairy cattle by calculation from values in milk. There seems little question but that dividing the ratio strontium-90/calcium in milk by 0·1 (typical value for observed ratio (milk/diet)) would give an estimate of dietary intake more simply and more accurately than would efforts to measure total intakes directly or to sum the strontium-90 and calcium contributions of components of the feed and water. Also, an estimate of the strontium-90/calcium being laid down in the newly forming and exchangeable bone of the animals could be obtained by dividing this ratio in milk by 0·5 (typical value for observed ratio (milk/plasma)).

It is well known that urinary calcium excretion is highly variable between individuals and even from day to day in the same individual. It is assumed that urinary strontium would also be variable. Since many of the factors that affect the behaviour of calcium also affect that of strontium in the same way, there is the likelihood that variations would be smoothed by using the strontium–calcium ratio as the basis of measurement.

Table 1 presents results from experiments with rats designed to show the variation of the observed ratio (urine/diet) with time following the start of ingestion. In Experiment 1, 8 rats of about 80 gm. body-weight were placed in metabolism cages and fed commercial rat pellets; the drinking water throughout the work was labelled with calcium-45 and strontium-85 for double-tracer studies according

Table 1. STRONTIUM–CALCIUM RELATIONSHIPS BETWEEN DIET AND URINE OF RATS

Experiment	Time after start of dosage	Observed ratio (urine/diet)*
1	17 hr.	2·6 ± 0·97†
	41 ,,	2·8 ± 0·72
	64 ,,	3·2 ± 0·55
	88 ,,	3·0 ± 0·52
	12 days	3·3 ± 0·53
	24 ,,	2·8 ± 0·28
	35 ,,	4·1 ± 0·20
		Average 3·1
2	14 days	3·2 ± 0·37‡

* Observed ratio = Sr^{90}/Ca^{40} in urine ÷ Sr^{90}/Ca^{40} in diet.
† Mean ± standard error of mean of 8 animals.
‡ Mean ± standard error of mean of 22 animals.

to usual procedures[6]. It is noted that a statistically constant value of observed ratio (urine/diet) had been reached by 17 hr. after the start of ingestion of the radionuclides. In Experiment 2, 22 rats were used ranging in body-weight from 80 to 500 gm.; a mean value is reported since no significant differences were noted between age-groups. This work shows that urine values in rats do reflect intake of the previous few days, and that the variability was not excessive as shown by the values for the standard error of the mean.

It is next of interest to examine the literature for values of observed ratio (urine/diet) for human beings in normal health. Two investigations have been reported in which the stable strontium and calcium of normal diets and urine were estimated[7,8]. From other work as yet unpublished, Dr. Herta Spencer of the Montefiore Hospital has made available some results of fall-out strontium-90 and stable calcium in the diets and urine of several individuals; in this series, three 6-day balance trials were run on each individual and the averages are reported. It is seen from Table 2 that, in 16 individuals of 4–64 years, the range of observed ratio (urine/diet) was 0·43–1·1 with a mean of 0·67 ± 0·058; the variability was about the same as in the work on rats. The average value of 0·67 is in agreement with the typical values of observed ratio (body/diet) about 0·25 and observed ratios (urine/plasma) about 3 (ref. 7). Thus an estimate of the strontium-90 to calcium of the total diet would be obtained from the measured strontium-90/calcium of the urine divided by the observed ratio (urine/diet), typical value 0·67 ; an estimate of the strontium-90 to calcium being laid down in the skeleton would be obtained by the strontium-90/calcium of the total diet multiplied by the observed ratio (body/diet), typical value 0·25.

Table 2. STRONTIUM–CALCIUM RELATIONSHIPS BETWEEN DIET AND URINE OF HUMAN BEINGS

Sex	Age	Method	Observed ratio (urine/diet)	Ref.
M	4 yr. 10 m.	(stable strontium and calcium in urine and diet)	1·0	8
F	9 yr. 9 m.		0·83	8
M	13 yr. 1 m.		0·60	8
M	14 yr. 4 m.		0·72	8
M	14 yr. 6 m.		0·78	8
F	29 yr.	(stable strontium and calcium in urine and diet)	0·45	7
F	31 yr.		0·59	7
M	40 yr.		0·50	7
M	45 yr.		0·50	7
M	50 yr.		0·40	7
F	18 yr.	(fall-out strontium-90 and stable calcium in urine and diet)	1·1	10
F	19 yr.		0·43	10
F	19 yr.		0·64	10
M	21 yr.		0·44	10
F	28 yr.		0·93	10
F	29 yr.		0·94	10
F	64 yr.		0·45	10
		Mean ± standard error of mean	0·67 ± 0·058	

Additional work is needed to get better measures of variability and of the effects of unusual diets, age or other factors that might modify the value of observed ratio (urine/diet). For example, it has been shown that new-born babies apparently have considerably higher values of observed ratio (urine/diet)[9]. In any event, it would appear feasible to collect enough urine samples (from the same individual and/or from different individuals) to give results of quite sufficient accuracy for purposes of food monitoring. The lower the contribution of food contamination relative to the total radiation exposure of the population, the lower the accuracy needed for assessment. Since present exposures from strontium-90 are only a few per cent of exposures from natural background, there would seem little need to evaluate food contamination with an accuracy much greater than appears to be possible from urinary surveys.

The advantages of using urinary assays are listed as follows : (*a*) the urinary samples reflect the total amount of strontium-90 and calcium that enters body tissues, and there is no need to guess the contributions of given food items or the availability of the strontium-90 in given food items; (*b*) values are calculated in terms of total strontium-90 and total calcium in the intake, which is probably the most important parameter governing the body burden ; (*c*) any sudden changes in levels of strontium-90 would be noted early, and the results could reflect any day-by-day variations; (*d*) sampling and bulking would be relatively simple and work could be designed to reflect individual intakes as well as group averages classed according to any category of interest ; (*e*) the results could be interpreted to give values for concentrations of strontium-90 and strontium-90/calcium ratios in areas of newly forming and exchangeable bone ; (*f*) from a continued programme of sampling it would be possible to construct the time pattern of radiostrontium-levels in bone.

Monitoring of food supplies by urinary assays would not eliminate the need for the examination of foods themselves, to obtain an understanding of the factors that govern the movement of radio-strontium in the food chain.

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Metabolism of the Sulphur Amino-Acids and of Sulphate in *Blattella germanica*

The cockroach and the Japanese beetle are able to utilize sulphate-S for the synthesis of methionine and cystine[1]. The German cockroach, *Blattella germanica* (L.), is also able to satisfy its sulphur requirements with homocystine, methionine sulphoxide, cysteic acid, taurine, and β-hydroxyethanesulphonic acid[2]. However, removal of the intracellular symbionts from the cockroach prevents incorporation of sulphate-S into cysteine and methionine[3].

The present communication describes the results obtained when ^{35}S-methionine, ^{35}S-cystine, and their oxidation products were administered to xenic (symbionts and intestinal micro-organisms present) and aposymbiotic *Blattella*. Further developments in the study of the metabolism of ^{35}S-sulphate are also described.

One to five microcuries of the ^{35}S-compound were injected into the insects, which were then maintained at 25° C. for one to seven days. They were then homogenized in 80 per cent v/v ethanol and the distribution of ^{35}S-components in the extracts and in hydrolysates of the residues was investigated. The results are summarized in Table 1.

The approximate distribution of the radioactivity in the known compounds is indicated by the number of plus signs. The results show that: (1) Cystine and cysteine were rapidly converted to glutathione, taurine, and sulphate by both xenic and aposymbiotic *Blattella*, but conversion of cysteine to methionine occurred only in xenic *Blattella*. (2) Cysteic acid was decarboxylated to taurine and oxidized, to a small extent, to sulphate by both xenic and aposymbiotic insects. (3) Most of the taurine is excreted unchanged. However, when intestinal bacteria are present, some of the taurine is degraded to sulphate, which may then be used by the xenic cockroach for the synthesis of cystine. (4) Sulphate sulphur is incorporated into cystine, methionine, taurine, sulphite, and various unidentified compounds by xenic *Blattella* but only sulphite and unidentified substances are formed by the aposymbiotic insect[3]. In contrast to the chick embryo xenic *Blattella* forms only a barely detectable amount of ^{35}S-taurine after $Na_2{}^{35}SO_4$ administration. This suggests that, in the cockroach, taurine is not formed directly from sulphate, but via cysteine. The reduction of sulphate to sulphite, which is widespread in plants and micro-organisms, occurs in the cockroach and, to our knowledge, only in the vitellin sac of the chick embryo[4]. (5) Methionine and methionine sulphoxide appear to be completely interconvertible by both xenic and aposymbiotic *Blattella*. A small quantity of CH_3SH, but no hydrogen sulphide or cysteic acid, was produced by the insects injected with methionine. (6) The sulphur of methionine sulphone was not detected in any other compound after its administration.

Cysteic acid has been proposed by a number of workers as an important intermediate in sulphur metabolism. As cysteic acid was not found in extracts of cockroaches after feeding or injection of sulphate, cystine, cysteine, methionine, or methionine sulphoxide, it does not seem to be a significant intermediate in *Blattaria*.

The results indicate that methionine and cystine are converted in the cockroach to sulphate by at least two pathways: (*a*) methionine sulphoxide ⇌ methionine → (homocysteine) → (cystathionine) → cysteine → (cysteinesulphinic-acid) → β-sulphinyl pyruvic acid → sulphite → sulphate; or (*b*) methionine → (homocysteine) → (cystathionine) → cysteine → (cysteinesulphinic-acid) → (2-aminoethane sulphinic acid) → taurine → ? → sulphate. Pathway *a* for the oxidation of cysteine is characteristic of most organisms and is probably the principal method of sulphate production in the cockroach.

In order to show that the results obtained with the aposymbiotic cockroaches were not due to traces of aureomycin carried over from the maternal diet, normal *Blattella* nymphs were placed on the aureomycin diet several days prior to cystine-^{35}S administration and caged in aureomycin-coated flasks. No inhibition of trans-sulphuration occurred as a result of this treatment.

The hypothesis that the intracellular symbionts are responsible for converting sulphate-S into methionine-S–cystine-S was substantiated by feeding *Blattella* nymphs a diet containing 0·1 per cent aureomycin until they matured. The adult insects, devoid of intestinal bacteria but retaining the intracellular symbionts, incorporated sulphur-35 from $Na_2{}^{35}SO_4$ into methionine, etc.; but their aposymbiotic progeny were unable to utilize sulphate for amino-acid synthesis even after re-establishment of the gut microflora.

Table 1. Radioactive Metabolites observed in the Alcoholic Extracts of *Blattella germanica* after Injection of Various Compounds labelled with Sulphur-35

Physiological condition of insect	Compound injected	Metabolites into which sulphur-35 was incorporated					
		Cysteine	Glutathione	Methionine + methionine sulphoxide	Sulphate	Taurine	Unidentified compounds
Xenic	Cyst(e)ine	+++	+++	+++	+++	++	Present
Aposymbiotic		+++	+++	–	+++	++	,,
Xenic	Cysteic acid	–	–	–	+	+++	Absent
Aposymbiotic		–	–	–	+	+++	,,
Xenic	Taurine	+	++	+	+	+++	Present
Aposymbiotic		–	–	–	+	+++	,,
Xenic	Sulphate	+++	+++	+++	+++	+	Present
Aposymbiotic		–	–	–	+	–	,,
Xenic	Methionine	+	+	+++	+++	–	Present
Aposymbiotic		+	+	+++	++	–	,,
Xenic	Methionine sulphoxide	+	+	+++	+++	–	Present
Aposymbiotic		+	+	+++	++	–	,,
Xenic	Methionine sulphone	–	–	–	–	–	Absent
Aposymbiotic		–	–	–	–	–	,,

The effect of temperature on sulphate utilization in xenic cockroaches was studied. Two unidentified compounds were produced in greater quantities at 5° C. while a third, which was almost absent at 5° C., accounted for about 10 per cent of the total radioactivity of the ethanol extract when the animals were maintained at 20 to 25° C. A chloroform extract of cockroaches which had been injected with sulphate-^{35}S and held at temperatures above 15° C. contained another unidentified radioactive compound, possibly a sulpholipid. Acid hydrolysis of this compound yields sulphate. Cystine and methionine were not formed at 5° C.

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PHYSIOLOGY

Selective Uptake of Specifically Bound Cobalt-58 Vitamin B_{12} by Human and Mouse Tumour Cells

MANY body fluids have been reported to contain substances that combine with vitamin B_{12}. However, research concerning the role of these substances in the absorption of vitamin B_{12} by animal cells has been confined mainly to work involving intrinsic factor preparations or normal human gastric juice. A large amount of evidence has led to general acceptance that vitamin B_{12} bound to intrinsic factor is taken up by intestinal mucosa cells to a greater extent than unbound vitamin B_{12} (ref. 1). Intrinsic factor also has been shown to promote uptake of vitamin B_{12} by rat liver slices[2,3] and liver homogenates[4]. The latter process apparently is not influenced by changes of temperature, and it is not affected by metabolic inhibitors such as 2,4-dinitrophenol[3].

The evidence that human serum may promote uptake of vitamin B_{12} by mammalian tissues remains contradictory. Herbert[5] has reported that the uptake of vitamin B_{12} by rat liver slices is enhanced if the slices are first incubated in the presence of human serum and then incubated in the presence of free vitamin B_{12}. Miller[2], however, was unable to demonstrate a stimulation of uptake of vitamin B_{12} upon incubating rat liver slices simultaneously with human serum and vitamin B_{12}. Several authors have reported that normal human serum may be required for the utilization of vitamin B_{12} by human erythroid cells in tissue culture[5,6], but others have not been able to confirm this finding[7]. We have shown that human serum caused marked stimulation of uptake of vitamin B_{12} by HeLa cells and mouse Ehrlich ascites tumour cells. Enhancement of uptake of vitamin B_{12} by Ehrlich ascites tumour cells was produced also by mouse ascites fluid. These observations led to the further work reported here.

Ehrlich ascites tumour cells were taken from *CF*1 mice seven or eight days after intraperitoneal transplantation. The cells were centrifuged and, after removal of the supernatant ascites fluid, were washed three or four times in cold Krebs–Ringer phosphate solution. The cells then were re-suspended in sufficient Krebs–Ringer solution to yield a 1 : 5 dilution; 0·5 ml. of this suspension per 5 ml. of incubation medium was used. The ascites fluid, filtered through cheesecloth after clotting had taken place, contained 1·8 mμgm., or less, vitamin B_{12} per ml. on assay with *Euglena gracilis*[1].

HeLa cells (generously supplied by Dr. A. M. Masson, Virology Laboratory, McGill University, Montreal) cultured in medium 199 (purchased from Microbiological Associates, Washington, D.C.) containing 20 per cent calf serum were collected six days after sub-culture by shaking in 0·045 *M* ethylenediamine tetraacetate at *p*H 7·4 for 30 min. The cells were washed three times in cold Krebs–Ringer phosphate solution containing 100 mgm. glucose per 100 ml. After re-suspension in Krebs–Ringer solution, approximately 0·2 ml. of packed cells was used per 6 ml. incubation medium. Human serum was obtained from patients with pernicious anæmia in relapse and from patients in the third trimester of pregnancy in order to ensure a low concentration of non-radioactive vitamin B_{12}. The sera used had a vitamin B_{12} content of 0·25 mμgm., or less, per ml., on assay with *Euglena gracilis*[8].

Hog intrinsic-factor concentrate (supplied by Ayerst, McKenna and Harrison, Ltd., Montreal) was optimally active in the Schilling test in a dosage of 100 mgm. per 0·5 μgm. vitamin B_{12}. Normal human gastric juice was obtained, after histamine stimulation, from at least six donors. The juice was filtered

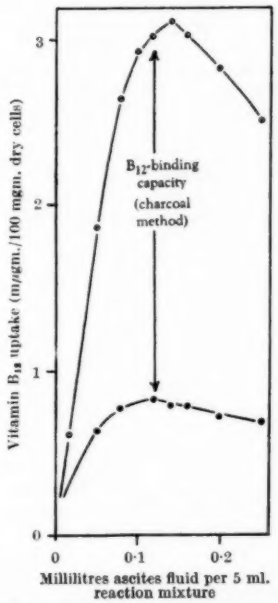

Fig. 1. Uptake of cobalt-58 vitamin B_{12} by Ehrlich ascites tumour cells with varying concentrations of ascites fluid. Vitamin B_{12}-binding capacity of ascites fluid by charcoal adsorption method was 5 mμgm. B_{12}/0·12 ml. ascites fluid. (5 ml. incubation medium contained 5 mμgm. cobalt-58 vitamin B_{12} and approximately 10 mgm. dry weight of cells.) ○—○, Uptake in 60 min. ●—●, uptake in less than 1 min.

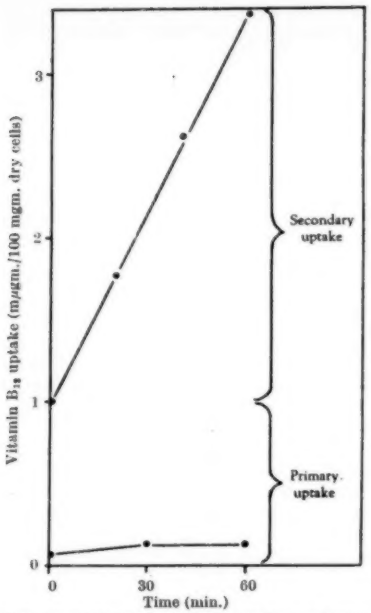

Fig. 2. Rate of uptake of cobalt-58 vitamin B_{12} by Ehrlich ascites tumour cells. (5 ml. incubation medium contained approximately 10 mgm. dry weight of cells.) ○—○, Medium contained 5 mμgm. cobalt-58 vitamin B_{12} and 0·12 ml. ascites fluid per 5 ml.; ●—●, medium contained 5 mμgm. cobalt-58 vitamin B_{12} per 5 ml., but no ascites fluid

through cheesecloth, adjusted to *p*H 10 to destroy pepsin, and neutralized to *p*H 7·0. The human serum, gastric juice, and mouse ascites fluid were stored separately in small aliquots at −20° C. until they were used.

The tumour cells were incubated in flasks containing 2–5 mμgm. cobalt-58 vitamin B_{12} (obtained from Merck of Canada), together with human serum or appropriate quantities of other B_{12}-binding materials, in the presence of Krebs–Ringer phosphate solution. Flasks were incubated at 37° C. in an atmosphere of air in a constant-temperature shaking water-bath. After an appropriate incubation period, the cells were removed by centrifugation and washed three times in cold phosphate-buffered saline. The cells were then resuspended in 1 ml. of water and counted in a well-type scintillation counter.

Ascites cells were incubated with a constant quantity of cobalt-58 vitamin B_{12} and varying amounts of ascites fluid and the amount of vitamin B_{12} taken up by the cells was estimated. The augmentation by ascites fluid of vitamin-B_{12} uptake by the cells was found to be optimal when the B_{12}-binding capacity of the ascites fluid added was equivalent to the quantity of vitamin B_{12} in the medium (Fig. 1). The B_{12}-binding capacity of ascites fluid, as determined by a modification of the charcoal adsorption method of Miller[2], was found to be 41·6 mμgm. vitamin B_{12} per ml. A similar relationship was observed between the charcoal-binding capacity of human serum and the uptake of cobalt-58 vitamin B_{12} by HeLa cells.

The pattern of uptake by ascites cells of bound cobalt-58 vitamin B_{12} is shown in Fig. 2. Negligible uptake values were obtained when ascites cells were incubated in the absence of ascites fluid. However, the addition of ascites fluid to the reaction mixture resulted in a very rapid initial uptake, occurring within the first minute of incubation, followed by a slower uptake directly proportional to time for at least 60 min. Further experiments showed that the initial or 'primary' uptake was almost completely unaffected by changes in temperature or *p*H, whereas the slower 'secondary' uptake was dependent on temperature and had a *p*H optimum at 7·4.

The effect of increasing concentrations of the ascites fluid–B_{12} on these two phases of uptake is shown in Fig. 3. Both describe exponential curves with increasing concentration. Similar observations were made with HeLa cells incubated in human serum.

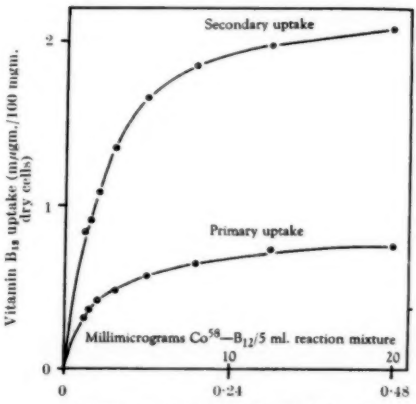

Fig. 3. Effect of varying concentrations of cobalt-58 vitamin B_{12} and ascites fluid on B_{12} uptake by Ehrlich ascites tumour cells. (5 ml. incubation medium contained approximately 10 mgm. dry weight of cells)

Both ascites cells and HeLa cells were found to take up the vitamin B_{12} only in the presence of certain fluids. HeLa cells took up vitamin B_{12} bound only to human serum, whereas vitamin-B_{12} uptake by ascites cells was stimulated by human serum, mouse ascites fluid, and mouse serum. Neither type of cell took up vitamin B_{12} bound to hog intrinsic-factor concentrate, human gastric juice, chick serum or calf serum. However, incubation with hog intrinsic-factor concentrate followed by incubation with cobalt-58 vitamin B_{12}, as described by Herbert[3], was effective in enhancing uptake by these cells: conversely, incubation with human serum or mouse ascites fluid followed by that of cobalt-58 vitamin B_{12} was not effective. This suggests that the phenomenon described here probably differs from that observed with rat liver slices by Miller[2] and Herbert[3].

In summary, mouse ascites fluid and human serum have been found to produce approximately a twenty-fold increase of uptake of vitamin B_{12} by mouse ascites tumour cells and by HeLa cells. Under the same conditions, human gastric juice and hog intrinsic-factor concentrate were ineffective in promoting uptake of vitamin B_{12}. Uptake of vitamin B_{12} occurring in the presence of mouse ascites fluid or human serum was found to be a bi-phasic reaction consisting of a rapid or 'primary' uptake which is probably of a physico-chemical nature, and a slower or 'secondary' uptake differing from the first in depending on temperature and possessing a *p*H optimum at 7·4.

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Outflux of Inorganic and Organic Phosphate during Membrane Depolarization of Excitable Tissues

IT was reported in a previous paper[1] that the outflux of phosphorus-32 from an isolated sartorius muscle of the frog into phosphate-free Ringer solution was accelerated markedly during electrical stimulation, or when the external solution was devoid of calcium or contained a high potassium (30 m*M*) concentration. Subsequent findings revealed that not only inorganic phosphate but also many organophosphates such as adenosine triphosphate (ATP) and phosphocreatine were released during membrane depolarization under such experimental conditions. The present communication concerns the outflux of nucleotides and other phosphates from frog sartorius muscle and spinal nerve roots during electrical stimulation and calcium depletion.

Isolated frog sartorius muscle was immersed in frog Ringer solution containing phosphorus-32 (carrier-free orthophosphate, 25 μc./ml.) for 2 hr. at room temperature (25° C.). In general, five paired muscles were used for each control and experimental run. The muscles were then washed in Ringer solution for 20 sec. and the initial soaking procedure continued for 2 hr., during which time the muscles were transferred every 20 min. into a series of beakers containing 10 ml. of phosphate-free Ringer solution. The extracellular phosphorus-32 was virtually washed out by this procedure[1]. The control muscles were then placed in 2 ml. Ringer solution (112 m*M* sodium chloride; 2 m*M* potassium chloride; 1·8 m*M* calcium chloride; and 2 m*M* sodium bicarbonate) and the experimental muscles in calcium-free Ringer solution (112 m*M* sodium chloride; 2 m*M* potassium chloride; 2 m*M* sodium bicarbonate; and 4 m*M* ethylenediamine tetraacetic acid (EDTA)) for a period of 1 hr. For stimulation experiments, the test muscles were immersed in Ringer for 40 min. while supramaximal electrical stimulation (1/3 sec. pulses) was applied through a pair of platinum electrodes dipped in Ringer solution. The final external solution was then run through a 'Dowex 1' anion-exchange column, and the phosphates separated and analysed as described elsewhere[2].

Two pairs of sciatic-spinal root nerve preparations were isolated from a bull-frog, and unilateral pairs used for test and control preparations. The nerve roots (ventral and dorsal) of the preparation were soaked in Ringer solution containing 50 μc./ml. of orthophosphate-^{32}P for 2 hr. (the sciatic nerves and spinal ganglia were kept exposed in a moist chamber during the whole course of the experiment). The nerves were washed and treated in the same manner described for the muscle preparations. The application of stimulation (50 pulses/sec.) and recording of action potential was carried out on the sciatic nerve for 40 min. The incubation medium was then analysed as in the case of muscle. The external solution contained phosphorus-32 originating from within the nerve fibre, while the phosphorus-32 of the interfibre was considered to diffuse out completely during the 2-hr. washing procedure (unpublished work).

Both the total amount and the specific activity of the tissue phosphates were studied in the external solution following incubation. In a calcium-free solution containing EDTA there was a considerable increase in all the phosphates examined, including

Table 1. SPECIFIC ACTIVITY AND AMOUNT OF PHOSPHATES LOST FROM FROG NERVE AND MUSCLE DURING ELECTRICAL STIMULATION AND IN CALCIUM-FREE EDTA

		Inorganic phosphate		Phospho-creatine		ATP		ADP		AMP		CMP		UMP	
		μmoles	*sa*	μmoles	*sa*	μmoles	*sa*	μmoles	*sa*	μmoles	*sa*	μmoles	*sa*	μmoles	*sa*
Calcium-free EDTA muscle	Control	0·04	280	0·01	50	0·02	40	0·005	—	0·008	—	0·005	—	0·00	—
	Exp.	0·15	270	0·13	60	0·04	50	0·01	10	0·02	2	0·01	1	Trace	—
	Change (per cent)	365	−6	770	20	100	25	100	—	250	—	100	—	—	—
Stimulated muscle	Control	0·03	250	0·01	45	0·02	40	0·00	—	0·00	—	0·00	—		
	Exp.	0·15	225	0·02	50	0·03	45	Trace	—	Trace	—	Trace	—		
	Change (per cent)	500	−10	100	11	150	12	—	—	—	—	—	—		
Calcium-free EDTA nerve	Control	0·05	210	0·005	45	0·01	35	0·00	—						
	Exp.	0·15	225	0·008	50	0·03	38	Trace	—						
	Change (per cent)	200	7	160	11	200									
Stimulated nerve	Control	0·04	205	0·005	40	0·01	30	0·00							
	Exp.	0·06	220	0·007	35	0·02	33	Trace							
	Change (per cent)	50													

ADP, adenosine diphosphate; AMP, adenosine monophosphate; CMP, cytidine monophosphate; UMP, uridine monophosphate. Each result is an average of 3 experiments agreeing within 10 per cent. *sa* = counts per min./μmoles phosphate × 10^{-3}

cytidylic and uridylic acid, the increase being greatest in inorganic phosphate and phosphocreatine. A small increase in the specific activity of phosphocreatine and ATP was observed, the activity in the other nucleotides being too low to compare. In the case of the electrically stimulated muscle, a 500 per cent increase occurred in inorganic phosphate, a 100 per cent increase in phosphocreatine, and 150 per cent in ATP. The other nucleotides, though showing a slight increase after stimulation, were present in only trace amounts. When frog spinal nerves were exposed to calcium-free EDTA solutions, a 200 per cent increase was noted in the concentration of inorganic phosphate, phosphocreatine and ATP, while the change in the specific activity of these fractions was insignificant. After electrical stimulation of frog nerves, a 50 per cent increase in the phosphorylated intermediates was observed.

The present results can be interpreted in a number of ways. The most likely interpretation is that there is a loss of calcium in excitable tissues exposed to either calcium-free EDTA solutions or electrical stimulation[2] This loss of calcium may result in a structural re-arrangement of the excitable membrane in such a way as to permit the loss of certain substances, either from the membrane itself or from the internal body of the tissue. The phosphates such as ATP may actually be intimately involved in the structural configuration of the membrane; and with the breakdown or loss of ATP there is a concomitant change in membrane permeability, as well as membrane depolarization. It is conceivable that the calcium diffuses out of the tissue as a phosphate complex, since, at the *p*H of the cell, calcium would be expected to form a complex readily with such phosphates as orthophosphate and ATP. The loss of phosphocreatine, however, would tend to indicate that some of the phosphates, at least, are not associated with calcium. To some extent, the efflux of phosphates from muscle may be due to the contractile events, but, in view of the results with nerve roots, it appears that to a considerable extent the efflux is associated with membrane depolarization. It has been suggested elsewhere[4] that the high-energy phosphates in excitable tissues may be compartmentalized, and that the important phosphates, so far as excitation is concerned, may be associated with the membrane itself. Preliminary work indicates that the specific activity of the phosphates in the external solution obtained during incubation is greater than that in internal muscle or nerve. It is possible, therefore, that the phosphates leaking out during loss of calcium are probably those primarily associated with the membrane, and that they may be more intimately related to the chemical events associated with excitatory phenomena.

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A Hydrostatic Function of the Accessory Respiratory Organs in Air-breathing Fishes

Accessory respiratory organs in fishes have generally been thought to be concerned with breathing atmospheric air[1]. That, like any other gas-filled space, they can also be connected with the perception of hydrostatic pressure has not been reported earlier. In fishes, perhaps the very existence of a swim-bladder, which takes the major role in regulating the density of the fish[2] and functioning as a hydrostatic organ[3], has diverted attention from other gas-filled organs which may have a similar function.

The accessory respiratory organs are supposed to have developed in response to exceptional environmental conditions which made aquatic respiration inefficient[4]. They may be simple secondary pharyngeal folds as in *Ophicephalus*, or sets of complicated and highly evolved opercular branchial chambers as in *Clarias*, *Heteropneustes* and *Trichogaster*[5]. In every case, fishes possessing these organs are known to visit the surface to gulp air[6].

When such air-breathing fishes are subjected to variations in hydrostatic pressure, control of the volume of air contained in the accessory respiratory organs occurs. At increased pressure the air in the accessory respiratory organ is retained, the fishes stay at or near the bottom and postpone their visit to the surface for relatively longer periods. When the pressure is decreased, the fishes begin to float up until they release gas from their accessory respiratory organs. Even in bottom-living fishes such as *Clarias* and *Heteropneustes*, a small reduction in pressure of 6–7 cm. mercury causes gas bubbles to be released. Further reduction brings about more release of gas, and when the pressure is reduced by 60–65 cm. mercury, practically all the gas from the accessory respiratory organs escapes. At such a reduced pressure, fishes like *Ophicephalus* and *Trichogaster*, which have a functional, physolistic type of swim-bladder[7], float helplessly upwards after the gas from the accessory respiratory organs has been expelled. Other fishes such as *Clarias* and *Heteropneustes*, which have a non-functional physostomatous type of swim-bladder[8], when put under reduced pressure, make dashing movements to the surface to gulp air, but being unable to retain it, eventually sink to the bottom. To ensure that in these physostome fishes the release of gas occurs from the accessory respiratory organs and not from the swim-bladder, further tests were made in *Heteropneustes* by puncturing its accessory respiratory organs. As the accessory respiratory organs in this fish are long, paired, sac-like structures situated dorsally, the puncturing operation could be made without causing undue injury to the fish. When such fishes were subjected to a reduction in pressure, the air was partly expelled through the punctured opening and partly from under the operculum.

The above experiments clearly show that there are striking similarities between the accessory respiratory organs and the physostomatous swim-bladder. In both cases the fish can control the volume of gas by swallowing and releasing air, and in this way either of them can act as a hydrostatic organ. It is interesting to note that physostome fishes predominate in fresh-waters[9] and so do the air-breathing fishes. In some forms such as *Clarias* and *Heteropneustes* the swim-bladder has become reduced and non-functional. These fishes possess large and highly developed

accessory respiratory organs presumably to compensate for the hydrostatic function of the swim-bladder as well as acting as respiratory organs. Such a correlation does not seem merely an accident, for in many fishes the swim-bladder acts as a respiratory organ[3]. It is therefore less surprising to find the accessory respiratory organ taking a part in the hydrostatic function.

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HÆMATOLOGY

Abnormal Fœtal Hæmoglobins

During the past few years, certain human fœtal hæmoglobin (Hb) variants characterized by electrophoretic mobility different from that of fœtal hæmoglobin have been described. They are: the Hb of Fessas and Papaspyrou[1], characterized by mobility at alkaline *p*H, faster than that of Hb *F*; Hb Bart's described by Ager and Lehmann[2], and also characterized by mobility at alkaline *p*H faster than that of Hb *F*; Hb Alexandra, slower than Hb *F*, described at the same time by Fessas *et al.*[3] and by Vella *et al.*[4].

The two fast hæmoglobins (Hb of Fessas and Papaspyrou and Hb Bart's) are most probably the same hæmoglobins. In fact, both these hæmoglobins have electrophoretic mobilities at alkaline *p*H between those of Hb *J* and Hb *H* (refs. 2–5) and at acid *p*H anodic mobility slightly lower than that of Hb *H*; their resistance to alkali denaturation is greater than that of Hb *A* but smaller than that of Hb *F* (refs. 2–5); their ultra-violet spectra are of the fœtal type (refs. 2–5), and in chromatography at *p*H 6 they have greater mobility than Hb *H* and any other type of hæmoglobin[2–5,6].

Hb Alexandra has been found rather frequently[6] in new-born Asiatic infants (Chinese, Malayans, etc.). Its mobility at alkaline *p*H is between that of Hb *S* and that of Hb *E* or A_2; it does not separate from Hb *F* on agar-gel electrophoresis at acid *p*H or on ion-exchange chromatography; its resistance to alkali denaturation and its ultra-violet spectrum are similar to those of Hb *F*; it gradually disappears during the first post-natal months.

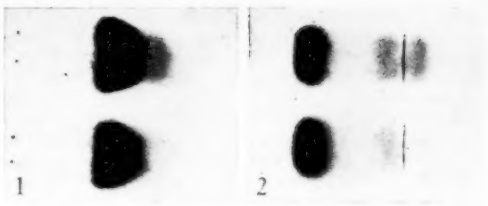

Fig. 1. Paper electrophoresis of Hb Bart's (399 *F*, above) and cord blood control (below). Glycine buffer *p*H 8·8, ionic strength 0·04. Staining with bromophenol blue. Greater mobility of fast fraction than of cord blood hæmoglobin

Fig. 2. Starch gel electrophoresis of Hb *H* (above) and Hb Bart's (390 *F*, below). Phosphate buffer *p*H 6, ionic strength 0·12. Staining with amido-Schwarz. ‡Identical mobility of faster fœtal fraction and Hb H_3 fraction

In a survey conducted among the Italian population on 1,200 samples of the umbilical cord blood of new-born infants, we have found two cases which had a fast fraction and one with a slow fraction.

The fast hæmoglobin has greater electrophoretic mobility at alkaline *p*H than that of the mixture of Hb *A* + Hb *F* (Fig. 1) and is very similar to that of fraction H_3 (the less fast of the two fractions present in carriers of Hb *H*, ref. 7). At acid *p*H, its anodic mobility is identical with that of fraction H_2 of Hb *H* carriers (Fig. 2).

Agar-gel electrophoresis at acid *p*H reveals between fraction *F* and fraction *A* two very small spots near each other which are, however, also found in umbilical cord hæmoglobins without a fast fraction, and which correspond probably to the two small fractions which normally precede Hb *A*.

On 'Amberlite *IRC*–50' column chromatography at *p*H 6, the fast fraction does not appear to be identifiable, probably due to the small quantity present.

Quantitatively (Table 1) the amount of fast Hb is small. The ultra-violet absorption spectrum of the isolated fast fraction is identical with that of Hb *F*; the alkali-resistance of the isolated fast fraction[8] is 4–5 min., which is intermediate between the resistance of Hb *A* and that of Hb *F*.

It is evident that the fast hæmoglobin has all the characteristics of Hb Bart's or Hb of Fessas and Papaspyrou. These characteristics are, however, identical also with those we found for H_2. In a previous survey[7] on 5 cases of Hb *H*-microcythæmia, we have shown the constant presence of two fractions of Hb *H*, one faster, Hb H_1, and one less fast, Hb H_2; and we have shown that Hb H_2 has a fœtal type of ultra-violet spectrum, whereas Hb H_1 has a normal

Table 1. Percentages and Spectrophotometric Index of the Various Hæmoglobin Fractions
Readings made on a Beckman *D.U.* spectrophotometer

Cases	Hb *a–r* per cent on the total Hb*	Values by starch block electrophoresis† (per cent)			$\frac{E\ (3{\cdot}000\ \text{Å.})}{E\ (2{\cdot}800\ \text{Å.})}$			$\frac{E\ (2{\cdot}800\ \text{Å.}}{E\ (4{\cdot}150\ \text{Å.})}$			$\frac{Ed^{\ddagger}}{E_0/2^{\S}}$		
		Fast Hb	Slow Hb	Hb *A* + Hb *F*	Fast Hb	Slow Hb	Hb *A* + Hb *F*	Fast Hb	Slow Hb	Hb *A* + Hb *F*	Fast Hb	Slow Hb	Hb *A* + Hb *F*
399 *F*	65·8	4·01		95·99	46·5		48·3	0·40		0·25	0·82		0·77
033 *F*	66·3	3·05		96·95	48·4		50·0	0·54		0·33	0·82		0·57
0153 *F*	60·9		13·05	86·95		45·9	45·5		0·31	0·25		0·74	0·56

* Hæmoglobin resistant to denaturation by alkali, according to Singer *et al.*
† According to Kunkel and Wallenius.
‡ Ed = E (5,800 Å.) of the solution at the end of denaturation by alkali.
§ E_0 = E (5,800 Å.) of the solution before denaturation by alkali.

Fig. 3. Starch gel electrophoresis of adult microcythæmic hæmoglobin (above), cord blood hæmoglobin with slow fraction (0153 *F*, at centre), normal cord blood hæmoglobin (below). Borate buffer *p*H 8·4, ionic strength 0·06. Staining with amido-schwarz. The slow fœtal fraction is faster than Hb A_2

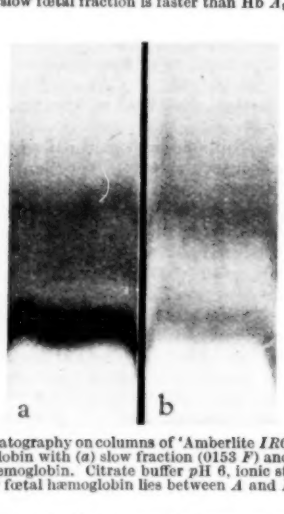

Fig. 4. Chromatography on columns of 'Amberlite *IRC*-50' of cord blood hæmoglobin with (*a*) slow fraction (0153 *F*) and (*b*) normal cord blood hæmoglobin. Citrate buffer *p*H 6, ionic strength 0·05. The slow fœtal hæmoglobin lies between *A* and *F* bands

spectrum. This finding has also been reported in the literature[9].

Hb Bart's and Hb H_2 have therefore the same electrophoretic and spectrophotometric behaviour (Table 1). It is now known[10] that Hb Bart's is a hæmoglobin composed of γ-chains only, that is, a fœtal hæmoglobin without α-chains, and Hb *H* (chromatographically separated)[11] consists of β-chains only, that is, it is an adult hæmoglobin without α-chains. It can therefore be deduced that Hb H_2 also consists of γ-chains only and Hb H_1 (corresponding to Hb *H* of the literature) of β-chains only: this would agree also with the spectrophotometric properties of these fractions.

The Hb of Fessas and Papaspyrou[1] and Hb Bart's[2] were originally described in microcythæmic new-born infants and later found to be present even after infancy; Tuchinda *et al.*[9b] and Boyo *et al.*[12], on the contrary, have reported disappearance of the fast fraction within three months after birth.

In the present two cases, an examination after three months showed that the fast fraction had disappeared. No trace of microcythæmia has been found in the children or their parents. The conclusion must be drawn that Hb Bart's, present at birth in non-microcythæmic individuals, disappears within a few months, whereas Hb Bart's, which appears in carriers of microcythæmic syndromes, persists.

The slow fœtal hæmoglobin, identified in one case during the survey, has a mobility at alkaline *p*H higher than that of Hb A_2 (Fig. 3) and lower than that of Hb *S*. On acid *p*H paper, starch-gel or agar-gel electrophoresis, it does not separate from Hb *F*. Chromatography reveals, in our hands, in tests extending over more than 36 hr., the separation of an intermediate band between that of Hb *F* and that of Hb *A* (Fig. 4) which does not appear in fœtal hæmoglobins without the slow fraction. This differs from the finding described in the literature for Hb Alexandra and requires confirmation.

A small amount of this slow hæmoglobin is also present (Table 1); it is always greater than the amount of fast fœtal Hb. The ultra-violet spectrum of the slow fraction isolated is of the fœtal type, but the alkali-resistance test also gives results for this hæmoglobin intermediate which are between those of Hb *F* and those of Hb *A*.

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Thalassæmia and the Abnormal Human Hæmoglobins

The preponderance of hæmoglobin *A* over hæmoglobin *S* in sickle-cell trait was observed in the course of our initial studies on the hæmoglobin of sickling cells[1]. Later work carried out in collaboration with other laboratories showed that the ratio of these hæmoglobins is inherited[2] and that simultaneous presence of a gene for an abnormal hæmoglobin and a gene for thalassæmia results in partial or complete suppression of synthesis[3,4] of hæmoglobin *A*. An exceptional case in which presence of a thalassæmia gene did not alter the proportion of hæmoglobins *A* and *C* in hæmoglobin *C* trait was observed elsewhere[5]. These findings formed the basic evidence on which we proposed the following concepts about the inheritance of hæmoglobin: (1) alleles may be present that control the synthesis of hæmoglobin *A* at

different rates[6]; (2) there may exist more than one locus for thalassæmia[7]; (3) in some cases thalassæmia and abnormal hæmoglobins may be controlled at the same locus[8]; (4) a structurally abnormal but electrophoretically normal hæmoglobin may be synthesized at sub-normal rate in thalassæmia[8,9].

The discovery[10] of a chemical abnormality in hæmoglobin *S* and the discovery[11] of two types of polypeptide chains in hæmoglobin *A* resulted in further additions to the theory of inheritance of hæmoglobin, namely, that control of the structure of hæmoglobin and control of its rate of synthesis are properties of the same allele, and that separate genetic control of synthesis of the two chains would result in four hæmoglobins in double heterozygotes; these postulates about the inheritance of thalassæmia and the abnormal hæmoglobins were given in a review[9] in 1957. The concepts that the alleles controlling the synthesis of myoglobin, adult hæmoglobin, fœtal hæmoglobin and hæmoglobin A_2 evolved from the same primitive allele and that comparative structural studies might reveal similarities in amino-acid sequence or position were proposed in the same review[9]. The presence of four hæmoglobins in double heterozygotes and the existence of structural analogies between hæmoglobin and myoglobin have been corroborated recently[12–16].

That chemical studies reported since 1957 have not substantially altered our inferences is evident from the conclusions expressed by Ingram and his associates in a recent series of papers[17–20]. These papers are remarkable for the extent to which the custom of giving pertinent references to the ideas and findings of others has been ignored.

An experimental test of our thalassæmia hypothesis by comparison with the amino-acid composition of peptides of hæmoglobin from normal human beings and patients with thalassæmia major was proposed by Ingram and Stretton[17]. We point out that thalassæmia minor, in which, according to our hypothesis, a mixture of normal adult and thalassæmia hæmoglobins is present, is a more readily available source of hæmoglobin than thalassæmia major, and might be used for this purpose. The observation of non-integral numbers of two amino-acid residues per molecule of hæmoglobin from a subject with thalassæmia minor would indicate the presence of an inherited variant with a single amino-acid substitution in the hæmoglobin of thalassæmia. The fractional deviation from the proper ratio of integral numbers would be proportional to the relative rates of synthesis of hæmoglobin *A* and thalassæmia hæmoglobin.

Familial differences in the ratio of hæmoglobin *A* to hæmoglobin *S* in sickle-cell trait were ascribed by one of us[9] to different alleles that result in electrophoretically indistinguishable but chemically distinct forms of hæmoglobin *A*. Accurate chemical analyses of the hæmoglobin *A* of sickle-cell-trait subjects with different inherited ratios would provide a test of this hypothesis. Furthermore, non-integral numbers of two amino-acid residues in the hæmoglobin of a normal individual would suggest heterozygosity in two inherited variants of hæmoglobin *A*.

Total amino-acid analyses of hæmoglobin or its constituent chains would fail to detect one type of alteration that has been suggested as a possible basis for electrophoretically identical subtypes, namely, the transposition of amino-acid residues in a chain[21]. Analysis of peptides obtained by tryptic digestion would also fail to detect transposition if the transposed segment did not contain a basic amino-acid. A search for such an alteration by complete sequential analyses of different samples of hæmoglobin *A* would be a formidable task for any laboratory. However, a number of laboratories are studying the amino-acid sequence of hæmoglobin *A*, with use of samples from different sources. If disagreements emerge with regard to the sequence of residues in polypeptide fragments of identical composition, inherited transpositions must be considered as a possible explanation.

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PATHOLOGY

Anaphylactoid Reaction in Rats

THE primary injection of dextran or egg white into rats produces hyperæmia, pruritus, and œdema of the face, tongue and paws; symptoms of the so-called anaphylactoid reaction[1]. While studying factors which modify this reaction, it became apparent that a proportion of rats failed to show this response. This problem was therefore examined in detail.

Most of the experiments were performed on groups of either male or female albino Wistar rats obtained from the Agricultural Research Council's Field Station at Compton. Their diet consisted of cubes (No. 41, Associated London Flour Millers, Ltd.) and they were allowed drinking water *ad lib.* The initial dose of dextran was 0·3 ml. per 100 gm. body-weight (180 mgm./kgm.). Fifteen out of 60 adult rats (25 per cent) failed to show the anaphylactoid reaction when this dose of dextran was given intraperitoneally, and a similar proportion did not react when the dextran was given intravenously together with Evan's blue dye. The animals initially resistant to this dose of

dextran were resistant to doses of 0·05, 0·2 and 0·8 ml. per 100 gm. when tested at weekly intervals. Similar results were obtained when fresh egg white (10 ml./kgm.) or a second brand of dextran was used. The rats which did not respond to dextran also failed to respond when subjected to stress (withdrawal of food for 24 hr.). They were not diabetic, and the weights of their adrenal glands were similar to those of control rats. There was no deficiency in the 5-hydroxytryptamine content of the skin of rats not responding to dextran, and dextran was ineffective in these rats even when pre-treated with 5-hydroxytryptophan. The intravenous toxicity of histamine was also unchanged.

Procedures which potentiate the anaphylactoid reaction in control rats were then tried in animals not responding to dextran. Pre-treatment of the rats with insulin (4 I.U./kgm.) or chlorpropamide (100 mgm./kgm.) failed to facilitate the response, and so did adrenalectomy when the animals were tested at 6, 13 and 20 days after the operation. In these experiments, up to 50 per cent of the control animals died after showing the anaphylactoid reaction. When insulin and the stress of food withdrawal were used in adrenalectomized rats, the resistant animals again failed to show the anaphylactoid reaction.

In this work, 832 rats have been used, and no fewer than 162 (about 19·5 per cent) have failed to respond to dextran or egg white. When, however, some of the experiments were repeated using Sprague Dawley and hooded Lister rats, the anaphylactoid reaction developed at a fast rate, and no failures were obtained. Further work is now in progress to determine the characteristics of the strain specificity with regard to dextran. The immunological aspects of the problem may be of paramount importance in the study of human allergies.

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Treatment of Thrombocytopænic Hæmorrhage with Œstriol Succinate

The therapeutic indications for œstriol have, until now, been limited to the treatment of superficial conditions of the cervix, vagina and vulva, and to the relief of menopausal symptoms. At the dosage-level effective in these cases, œstriol has no effect on the endometrium and does not, therefore, lead to irregularities in the menstrual cycle or to uterine hæmorrhage.

I have recently carried out an investigation into the hæmostatic properties of a pure intravenous preparation of œstriol succinate (kindly supplied by N.V. Organon, Oss, Holland) in hæmorrhages of varying pathogenesis. It became clear very early in our investigations that this substance has a rapid effect on all types of thrombocytopænic bleeding, its action being particularly marked in the acute toxic and allergic thrombocytopænias.

The substance was administered to patients of normal weight in intravenous doses of 10 mgm. and provided adequate protection from hæmorrhages for about 48 hr. None of the 100 patients so far treated in this way has shown side-effects.

My investigations into the mechanism of the normal clotting process have demonstrated that œstriol succinate acts on this mechanism in two distinct ways: (1), a direct action on the blood vessels, reducing their permeability; and (2), an effect closely similar to that of thrombocyte factor 3. The latter action can be irrefutably demonstrated using the thrombokinase-formation test. Details of my work on the clotting mechanism will be published elsewhere.

No significant increase in thrombocyte count was observed during the above treatment. The dangerous incidents which occur all too frequently in the course of thrombocytopænia can, nevertheless, be rapidly and economically controlled in this way until such time as a more normal thrombocyte count can be restored by other means. I have had frequent recourse to œstriol succinate in cases with symptomatic thrombocytopænia consequent on leukosis, panmyelopathy and overdosage of cytotoxic agents. In addition, hæmorrhages following overdosage with anticoagulants, as well as post-traumatic bleeding in patients on anticoagulant therapy, can be better controlled when œstriol succinate is administered than on vitamin K therapy alone. This effect must be attributed to the direct vascular action of the preparation. Œstriol succinate, in view of its high therapeutic efficiency and its very good tolerance, has proved to have a wide field of application in this hospital, and one must hope that it will become generally available in the near future.

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Effect of Enzyme Injections on Mitosis in Regenerating Liver

The addition of deoxyribonuclease to tissue-culture media affected mitosis in tissue cultures[1,2]. In mice bearing Ehrlich ascites tumours, injections of the enzyme decreased the incidence of mitoses in the tumour cells and increased the survival-time of the animals[3]. Similarly, injections of xanthine oxidase and ribonuclease affected the rate of tumour growth and the survival time[4–6].

In view of these facts, it has been thought of interest to study the effect of deoxyribonuclease (DNase), ribonuclease (RNase) and xanthine oxidase on a rapidly dividing normal tissue such as regenerating liver following partial hepatectomy. (Deoxyribonuclease and ribonuclease were purchased from Worthington Biochemical Corporation, Freehold, New Jersey. The xanthine oxidase was a gift from Prof. F. Bergel, Chester Beatty Research Institute, London.)

Wistar rats of average weight 218 gm. (194–242 gm.) were partially hepatectomized (66 per cent) according to the method of Higgins and Anderson[7] between 9.30 and 11.30 a.m. to eliminate the variations due to the periodicity of mitotic activity[8]. The hepatectomized rats were injected intraperitoneally with sodium chloride 0·85 per cent or solutions of albumin, DNase, RNase (5 mgm./ml.), or xanthine oxidase in sodium chloride 0·85 per cent. The original solution of xanthine oxidase prepared in phosphate buffer containing sodium salicylate and ethylenediamine tetraacetic acid as preservatives

Table 1. Effect of Enzyme Injections on Mitosis in Regenerating Rat Liver

Group	No. of rats	Mitoses No.	Prophases No.	Prophases Per cent	Metaphases No.	Metaphases Per cent	Anaphases No.	Anaphases Per cent	Telophases No.	Telophases Per cent
Controls*	6	4·7 ± 0·2	1·8 ± 0·1	38·4 ± 1·7	2·0 ± 0·2	41·8 ± 2·6	0·4 ± 0·1	9·0 ± 0·9	0·5 ± 0·1	10·8 ± 0·6
DNase (*A*)	5	4·3 ± 1·4	1·0 ± 0·3	24·2† ± 1·2	2·5 ± 0·8	59·7† ± 3·2	0·4 ± 0·2	8·2 ± 2·7	0·4 ± 0·2	7·9 ± 1·7
DNase (*B*)	4	4·0 ± 0·4	1·0 ± 0·1	24·2† ± 3·3	2·3 ± 0·4	58·0† ± 5·2	0·3 ± 0·1	6·6 ± 1·6	0·4 ± 0·1	11·2 ± 2·1
Xanthine oxidase (*A*)	5	3·4 ± 1·4	0·7 ± 0·3	19·4† ± 2·2	2·1 ± 0·7	61·4† ± 2·0	0·3 ± 0·1	8·7 ± 2·2	0·3 ± 0·2	10·5 ± 1·7
Xanthine oxidase (*B*)	4	4·1 ± 2·5	1·1 ± 0·5	26·9† ± 1·8	2·1 ± 1·0	52·1† ± 2·0	0·4 ± 0·3	9·1 ± 2·6	0·5 ± 0·3	11·9 ± 2·0
RNase (*A*)	3	4·3 ± 1·4	1·9 ± 0·7	43·0 ± 2·3	1·9 ± 0·7	43·5 ± 3·9	0·2 ± 0·1	6·6 ± 3·0	0·3 ± 0·1	6·9 ± 1·2

* Controls were injected intraperitoneally with 1 ml. sodium chloride 0·85 per cent or albumin (5 mgm./ml.) immediately after the hepatectomy. Groups DNase, xanthine oxidase and RNase (*A*) were injected intraperitoneally with 1 ml. of enzyme (5 mgm./ml.) immediately after the hepatectomy. Groups DNase and xanthine oxidase (*B*) were injected intraperitoneally with 1 ml. of enzyme (5 mgm./ml.) at 6 and 24 hr. after the hepatectomy.

† Statistically significant at a level better than 99·9 per cent.

was dialysed against two litres of sodium chloride 0·85 per cent during seventy-two hours in the cold. After dialysis, the enzyme activity was determined by a standard method[9] and the activity corresponded to the destruction of 1·27 μmole of xanthine per min. per ml. of solution.

Forty-eight hours after hepatectomy, the rats were killed by decapitation and the excised liver fixed in neutral 10 per cent formaldehyde. Histological sections were prepared by the usual method and stained with eosin and hæmatoxylin.

The incidence of mitotic figures in liver parenchyma was determined by differential counts. A reticule delimiting a square field was placed in one of the oculars of a binocular microscope and this field was placed at random over the histological section. The nuclei present in the field were examined under immersion and differential counts were made of resting cells, prophases, metaphases, anaphases and telophases. When all the nuclei present in a field had been examined, the counts were carried out in similar fields taken at random until at least 6,000 nuclei were counted. The results were expressed as the number of mitotic figures per hundred cells.

Table 1 shows the results obtained for the incidence of mitosis and mitotic phases in regenerating liver following the injection of control or enzyme solutions. The proportions of the mitotic phases are also included. From the results, it would seem that the mitotic activity was not much affected by enzyme injections. The largest variation was observed in the group xanthine oxidase (*A*) injected with a single dose immediately after hepatectomy and was of the order of 30 per cent. The incidence of prophases, on the other hand, was much affected in rats injected with DNase or xanthine oxidase. Decreases from 1·8 to 1·0, 1·0, 0·7, and 1·1 respectively were observed. The incidences of metaphases, anaphases and telophases, however, varied only slightly. The important decrease in the incidence of prophases became masked in the values obtained for the incidence of mitosis because it represents only a fraction of the mitotic activity.

The decreases in the number of prophases were reflected by significant decreases in the proportions of prophases relative to the other phases of the mitotic cycle. Significant increases in the proportions of metaphases were consequently observed. The injection of RNase did not affect the number of the various mitotic phases or their proportions.

Table 2 shows the most important cytological abnormalities observed in the mitotic figures. The control rats showed a few pycnotic metaphases as well as a few metaphases with one or two lagging chromosomes. These abnormalities were very frequent in the animals treated with DNase. In these animals it was also frequently noticed that chromosomes were lagging to join the two sets at anaphase and chromosomal bridges were present. In the animals treated with xanthine oxidase, pycnotic metaphases as well as colchicine-like metaphases were frequently observed.

Table 2. Cytological Abnormalities

	Control	DNase
Chromosomes lagging to reach the equatorial plate in metaphase	rare, one or two chromosomes lagging	very frequent, many chromosomes lagging
Pycnotic metaphase	rare,	frequent,
Chromosomes lagging to join the two sets in anaphase	nil	frequent
Bridges of chromosomes in anaphase	nil	present
	Control	Xanthine oxidase
Colchicine-like metaphase	nil	frequent

From the results described it would seem that the duration of prophase has been shortened by the injection of DNase and xanthine oxidase as indicated by the significant lowering of the incidence of prophases. The metaphase and anaphase seemed also to be affected to a certain extent as shown by the abnormalities noted.

We acknowledge the help of Dr. Roger Boothroyd, of McGill University, in the classification of the mitotic abnormalities. Dr. Roger Daoust has been most helpful by his advice on differential counts. The work has been supported by grants to Dr. A. Cantero, director of the Research Laboratories, from the National Cancer Institute of Canada.

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BIOLOGY

Myopathy affecting the Rottnest Quokka (*Setonix brachyurus*) reversed by α-Tocopherol

The quokka (*Setonix brachyurus*) is a small marsupial about the size of a fox terrier which inhabits Rottnest, an island lying in the Perth Basin ten miles from the coast of Western Australia. In recent years the species has been extensively studied by the Department of Zoology of the University of Western Australia, as the potential offered by the animal for ecological research is unique.

For several years in Perth and overseas, paralysis of the hind limbs has been observed in the quokka while in captivity. This disease has hitherto ended fatally despite various therapeutic measures including antibiotics and antihelminthics. It is characterized by weakness of the hind limbs which begins insidiously but progresses rapidly to complete paralysis. There is marked wasting of the muscles of the pelvic girdle and the disease invariably terminates in death in a few days to several weeks. At autopsy pallor and atrophy of the pelvic and femoral groups of muscles are found. On histological examination of affected muscles individual fibres are swollen, fragmented and show variability of cytoplasmic staining. Many fibres are without nuclei, and in others there is pycnosis. There are proliferations of sarcolemmal cells and infiltrations of variable cellularity of lymphocytes, plasma cells and polymorphs. Metazoan parasites are not found.

Early in 1960 representative slides of the muscle lesion were sent to Dr. Raymond Adams, Bullard professor of neuropathology, Harvard Medical School, for his opinion. He replied that the cause of the paralysis and of the lesions observed might well be due to deficiency of vitamin E in the diet. Dr. ten Seldam, professor of pathology, University of Western Australia, came to the same conclusion when investigating other similar material for Dr. S. Barker of the Zoology Department. Accordingly experiments were undertaken to test the effects of vitamin E administration to paralysed animals.

Ten quokkas which developed paralysis while in captivity were selected for trial treatment. These animals were from several groups which were maintained on a standard diet of high protein (17–21 per cent) 'sheep cubes' consisting of bran, pollard, oat meal, linseed meal, whale meal, molasses, urea and mineral salts. This diet was supplemented with bread and green vegetables including lucerne, lettuce, spinach and grasses growing in the vicinity. Nine of the treated animals were given the vitamin orally in doses ranging from 200 to 600 mgm. over several days. In all these animals there was rapid and complete clinical recovery in two to three weeks. Only one of the ten animals died. It was moribund

Table 1

Date	Quokka	Urine (vol. 24 hr.)	Creatine (24 hr.)	Creatinine (24 hr.)
27.10.60	6	27 ml.	24 mgm.	0·010 gm.
28.10.60	7	48 ml.	27 mgm.	0·008 gm.
2.11.60	8	92 ml.	58 mgm.	0·011 gm.
13.1.61	6	48 ml.	15·8 mgm.	0·03 gm.
13.1.61	7	67 ml.	36·8 mgm.	0·057 gm.
13.1.61	8	96 ml.	24·0 mgm.	0·079 gm.

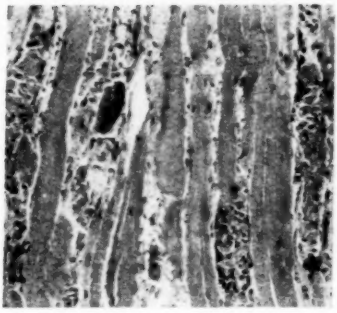

Fig. 1. Muscle biopsy of quokka 5. Original biopsy, October 17, 1960. Pronounced degenerative changes. (Hæmatoxylin and eosin × 78)

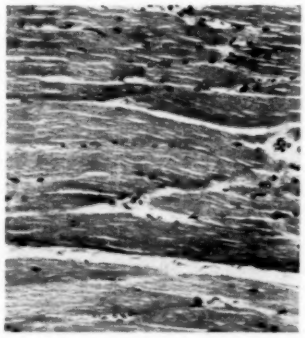

Fig. 2. Muscle biopsy of quokka 5. Second biopsy November 21, 1960. Restoration to normal. (Hæmatoxylin and eosin × 78)

at the beginning of the experiment and failed to respond to the vitamin given by injection.

Muscle biopsies were taken on the first or the second day of treatment. In the nine animals which recovered this first biopsy showed histological changes similar to those depicted in Fig. 1. In seven animals subsequent biopsy was performed at a site close to the original. These biopsies were taken from 1–8 weeks after clinical recovery. Two of the second biopsies showed the muscle to have completely recovered. In four others there was still minimal evidence of degeneration (Fig. 2), and in one no improvement of the lesion was observed notwithstanding the clinical recovery. In general, the longer the period was between biopsies the greater the observed improvement was in the histological appearances. 24-hr. samples of urine for creatine and creatinine estimations were taken from three of the animals at the time of the original biopsy and again after full recovery. The results, which support the clinical and biopsy findings, are given in Table 1.

Deprivation of vitamin E in the diet of the quokka is at present being tried in a small series of animals and the early results suggest that the lesion can be induced in this manner. No control animals have died naturally or have shown evidence of disease.

Grateful acknowledgment is made to Prof. ten Seldam, who suggested this work, and for the advice and technical assistance given by Dr. I. Kaldor (Department of Physiology), and members of the Department of Zoology of the University of Western Australia. Mr. H. Upenieks, medical illustrator.

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Structure and Action of the Sucker of *Echeneis*

Fig. 1 is a diagram of the sucker of *Echeneis* taken after staining it with alizarin red and rendering it transparent in glycerin. This reveals the presence of a chamber below the posterior shield of the sucker, about which no mention has so far been made in the literature. Hora[1], who at first doubted whether the sucker was a real sucker, later was convinced that the outer rim functioned as a sucker and that there were smaller paired suckers as well; Sewell's[3] observations confirmed this.

My observations show: (1) That in addition to the paired chambers there is an unpaired chamber below the posterior shield which has no opening on the dorsal side. (2) While all the other paired chambers are filled with sea water when the fish is swimming about, this chamber also contains some fluid but not sea water as there appears to be no connexion with the exterior. (3) At the posterior end of the chamber there is a pear-shaped hollow bony structure with one or more openings in it and there are two channels one on either side of it suggesting that by its movement forward and backward it can act as a valve regulating the flow of liquid in the chamber. (4) The entire base of the disk is paved with bone consisting of plates overlapping each other and with two pairs of holes in them. (5) The laminæ carry transverse bars against which the bases of the teeth abut, forming effective buttresses. (6) A pair of large hooks from the buttress interlock with the holes in the basal plates. (7) The pear-shaped, hollow, bony structure becomes completely detached when teased in caustic potash.

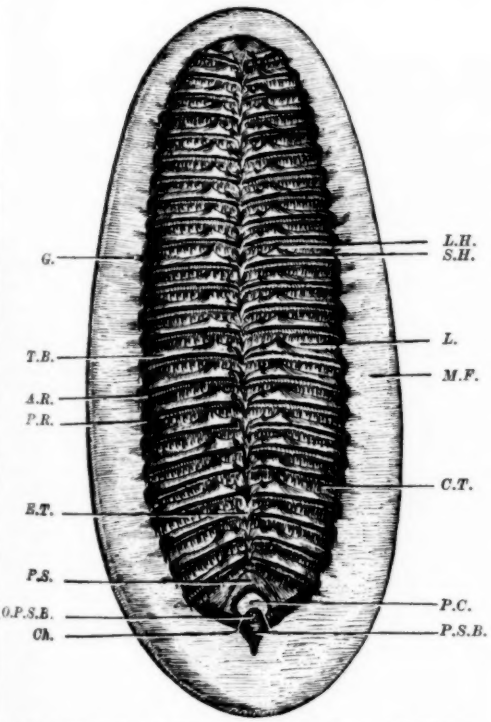

Fig. 1. The sucker of *Echeneis* as seen when stained in alizarin red and rendered transparent in glycerin. *A.R.*, anterior ridge; *Ch.*, channel; *C.T.*, comb-like teeth in three rows; *E.T.*, enlarged tooth pointing backwards; *G.*, glyph or spillway; *L.*, lamella between ridges; *L.H.*, large hook which fits into basal plate below; *M.F.*, marginal flap; *O.P.S.B.*, opening in pear-shaped body; *P.C.*, posterior chamber; *P.R.*, posterior ridge; *P.S.*, posterior shield; *P.S.B.*, pear-shaped body (valve); *S.H.*, small hook which fits into basal plate below; *T.B.*, transverse bar or buttress

The attachment presumably takes place in the following stages: (1) the marginal flap is the first to adhere; (2) the sea water is next sent out of the paired pouches from below the posterior ridge of each lamella; (3) next the interlocking of the lamellæ with the basal plates takes place; (4) any remaining water escapes by the spillways resembling siphonoglyphs; (5) the posterior shield is the last to adhere when the body fluid in the median chamber is withdrawn.

The release of the disk can be brought about by admitting sea water artificially: that is, by inserting sticks, strings or the blade of a penknife, but in Nature the processes mentioned above work in reverse. The posterior shield is the first to be released by the admission of body fluid into the chamber below it. Sea water now enters the space between the shield and the surface of adhesion and is carried to the siphonoglyphs, through which it enters, bringing about the release of the hooks. Water now is able to enter into the paired chambers, producing the "hissing sound" mentioned by Hora[2] and the "slight sucking noise" of Sewell[3]. During the process the water is strained free of all solid particles which might be floating in the sea water by the comb-like teeth. As each chamber is partly overlapped by the next a set of chain reactions occur so that all the paired pouches are progressively filled with sea water and the entire disk released.

Sewell mentions that when *Echeneis* is attached to any object all the movements of the fish except those of the mouth and gills seem to be suspended, and Norman[4], quoting it, says a detailed study of the nervous mechanism involved would be of interest. I venture to suggest that the movement of the body fluid in and out of the posterior chamber is obviously responsible for the setting of the nervous mechanism to work.

A detailed description of the sucker is in preparation, and it is expected to show that the sucker instead of being a partial vacuum is really a mechanism precisely built and as efficient as any that human ingenuity can invent, in which case the sucking power must be 15 lb./sq. in. As some of the suckers cover an area of more than 8 sq. in., the fish, theoretically at least, must be able to suck with a power exceeding 100 lb.

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The Path of the Giant Cell Axons in *Aplysia depilans*

The presence of large nerve cells in the abdominal ganglion of *Aplysia* has provided a valuable preparation for the study of the physiology of invertebrate neurones[1–8]. The largest cell is usually referred to as the giant nerve cell[1–3,6–8] and it may be 300–800μ in diameter. It is excited synaptically by pathways entering most, if not all, of the nerves of this ganglion, but its only efferent axon passes anteriorly along the right pleuro-visceral connective. As almost all the work on the abdominal ganglion has been done with it completely isolated from the rest of the nervous system, the further path of this axon and the possible functions of this very large cell have not been understood. Experiments, which included the insertion of micro-electrodes into this giant cell when the abdominal ganglion retained its functional connexions with the rest of the nervous system in a whole animal preparation, have provided evidence for the branching of this axon and have excluded certain possibilities concerning the function of the giant cell.

Fig. 1 summarizes diagrammatically the results of several different types of experiment which have confirmed one another. The axon of the giant cell after passing to the right pleural ganglion divides into branches which enter each of the main nerves innervating the right parapodium and the right side of the foot. Another branch continues forwards in the cerebro-pleural connective and through the cerebral ganglia to the left pleural ganglion. Exactly where the branching occurs is not certain; but, from the evidence available, that shown in the diagram seems the most probable. Electrical stimulation of any of the nerves containing a branch of this axon produces an impulse in each of the other branches. In these conditions, an antidromic spike is recorded in the giant cell following the passage of an impulse down the right pleuro-visceral connective. However, transmission between the different branches of this neurone is not always readily achieved, and repetitive stimulation is sometimes necessary. On the other hand, transmission often fails at relatively low frequencies. When this happens, however, it is not invariably preceded by a gradual lengthening of the delay which might be expected if a synaptic process were involved. Furthermore, delay between the application of the stimulus to one branch of the neurone and its appearance at another point is the same, regardless of the direction of impulse conduction. It may be assumed, therefore, that the failure of transmission in an antidromic direction is due to the low safety factor at the branching, especially as the more distant axonal branches are smaller than the main axon. A failure to conduct an impulse from the main axon to the branches in the orthodromic direction was never observed. Further confirmation was obtained by stimulating the giant cell itself by depolarizing the soma membrane and recording impulses with constant delays in the different nerves to which it sends a branch. In addition it is clear that, in spite of the axon crossing several ganglia, this cell is only excited synaptically in the abdominal ganglion. Different natural stimuli to an intact animal have never elicited a spike from a part of this neurone other than that contained in the abdominal ganglion.

Another giant cell has now been discovered on the left side of the animal. Work similar to that outlined above showed an almost identical pattern

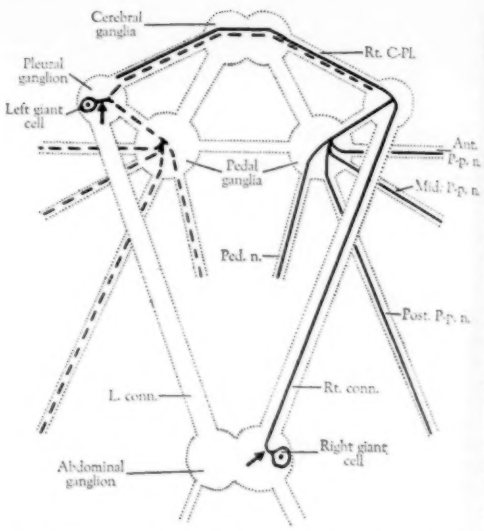

Fig. 1. Diagram of the path of the two giant cell axons in the nervous sytem of *Aplysia depilans*. Right cell, full lines; left cell, dashed lines. Arrows indicate the region where the giant neurones are excited synaptically. *Rt. C-Pl.*, right cerebro-pleural connective; *Rt. conn.*, right 'pleuro-visceral' connective; *Ant.*, *Mid.*, and *Post P-p. n.*, anterior, middle and posterior parapodial nerves; *Ped. n.*, pedal nerve

of branching (Fig. 1). The main difference is that in this case the cell body lies in the pleural ganglion and not in the abdominal ganglion. However, if the long axon in the right pleuro-visceral connective were shortened a symmetrical arrangement would result.

The precise homologies of the ganglia associated with the visceral loop in *Aplysia* are incompletely understood. This is mainly due to the absence of developmental evidence, and present views are based largely upon considerations of the external morphology of the central nervous system. The discovery that the largest cell on the two sides has basically the same distribution suggests the tempting hypothesis that they are homologous and that the cell bodies reside in different ganglia as a result of the complex morphogenetic changes which occur during torsion. In an idealized gastropod the visceral loop has along its length two parietal ganglia and a single or double visceral ganglion. If, following torsion, the right parietal ganglion became fused with the visceral ganglion, whereas the left ganglion or some part of it had remained fused with the left pleural ganglion, the difference in position of these two cells would be explained. Fusion of the right parietal ganglion (supra-intestinal) with the visceral ganglion is generally accepted, but the fate of the left parietal ganglion has not been established[9].

Little information has been obtained concerning the function of the left giant cell, because of the difficulty of recording from this cell in the intact preparation. Activity of the right giant cell has been recorded intracellularly in many preparations, and a study made of the conditions in which it becomes active. This occurs when mechanical stimulation is applied to any part of the body, with the head and gill region being the most sensitive. Excitation of the cell by either depolarizing currents passed through an intra-cellular micro-electrode or direct mechanical stimulation has not consistently evoked any overt

movement of the animal. This disposes of the possibility that this cell is directly involved in the dorsal folding movements of the parapodia which occur when the animal is prodded and seem to represent a form of escape reaction. Nevertheless, during such movements of the parapodia which occur spontaneously in some preparations, the giant cell nearly always fires and it seems to be associated with this response although many other neurones in the abdominal ganglion do the same. It is clear that the giant cell does not have a function similar to the giant efferent neurones of other animals. Recordings were made from single neurones of the right pleural and pedal ganglia during stimulation of the giant cell and the abdominal ganglion. No evidence has been obtained for the existence of synaptic connexions between the giant cell axon and the cells which were sampled in several preparations. The possibility that this cell has an effect on the intensity or threshold of this defence response or other activities in the animal is difficult to investigate and remains a possible function of this very large cell.

This work was carried out in the Marine Biological Institute of Arcachon, France.

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Maxillary Index in *Anopheles gambiae* Giles

Freshwater *Anopheles gambiae* may be distinguished as two co-existing biological races[1–3], on the basis of their maxillary indices, namely: (i) a paucidentate race, breeding in temporary pools of low organic content, which is anthropophilic and mainly exophilic, and (ii) a multidentate race, breeding in permanent types of water of high organic content, which is zoophilic and endophilic. Others[4–7] hold the opposite view, that the maxillary index is not a precise enough character for demonstrating this distinction. The present observations are in agreement with the reported breeding preferences of paucidentate and multidentate populations, but they also support the conclusion in ref. 6 that the larval environment has a powerful influence on the maxillary dentition.

Observations were made on three populations of *A. gambiae* (I, II and III), in the neighbourhood of Kampala, in Uganda, East Africa. Population I bred in a large temporary pond, on the Kampala-Hoima Road; II, in small, temporary, rain puddles, in an old road passing through Nakazi Swamp, on the Kampala-Bombo Road; and III, in permanent water, in the more exposed parts of Nakazi Swamp. Population I occurred some 10 miles from both II and III; while II and III were only 10–50 yards apart. The maxillary indices of the three populations are summarized in Table 1.

Table 1. Maxillary Indices (M.I.) of the Three Populations

Maxillary Index	Mosquito population and frequency (per cent)		
	I	II	III
9·5	2·5		
10·0	5·5		
10·5	12·5		
11·0	10·5	1·0	
11·5	27·5	3·0	
12·0	13·5	6·5	2·0
12·5	17·0	13·0	1·5
13·0	3·5	19·0	4·5
13·5	6·0	24·0	11·5
14·0	0·5	15·0	17·0
14·5	1·0	13·5	25·0
15·0		5·0	9·5
15·5			11·5
16·0			6·0
16·5			8·5
17·0			2·0
17·5			1·0
No. mosquitoes dissected	200	200	200
Mean M.I.	11·64	13·38	14·66

Table 1 shows that population III had a higher maxillary index than either I or II; thereby supporting the conclusion reached by other workers[1–3] that multidentate populations tend to breed in permanent waters and the paucidentate in temporary waters. In this respect, the distinction between the maxillary dentition of II and III is particularly striking, in view of the close proximity of their breeding places. In such circumstances, genetic distinctiveness could not be maintained, unless there were sexual isolation between the two populations.

The difference in the maxillary dentition between populations I and II is also particularly interesting. The breeding water of I was less temporary, and, presumably, had a higher organic content, than that of II. It would, therefore, be expected that population I should have a higher maxillary index than II. The results (Table 1) show the reverse. The explanation of this anomaly may be found in the spatial relations of the three populations. If, as it has been suggested[1–3], we are dealing here with two co-existing biological races, the close proximity of the breeding places and, consequently, of the adult populations may have resulted in the production of a hybrid population (II) with an intermediate maxillary index. The distance between temporary and permanent breeding places may, therefore, affect significantly the difference between the maxillary indices of the respective mosquito populations.

That the maxillary index may be increased by increasing artificially the temperature of the breeding water is shown in Table 2. These results agree with those in ref. 6. However, the significance of these results, in relation to what happens in the field, is not obvious. The vast majority of *A. gambiae* breeding places are exposed, temporary, shallow pools, in which the water temperatures are often considerably much higher than in the less favourable, permanent-water habitats. Yet, the maxillary index is higher in the latter than in the former. It is probable that this discrepancy is due to the fact that the artificially high laboratory temperatures are made continuous; while those in the field occur for only a short period of the day and thus they fluctuate with the daily rhythm. In this respect, a study of the maxillary index of *A. gambiae* breeding in continuously high temperatures, in tepid water from hot springs[8], would be particularly worth while.

Table 2. Maxillary Index (M.I.) of Population I Bred in Field Water at Constant High Temperature (32° C.) in the Laboratory

Maxillary Index	Frequency (per cent)
13·5	1·7
14·0	12·1
14·5	20·7
15·0	15·5
15·5	20·7
16·0	12·1
16·5	8·6
17·0	5·2
17·5	1·7
18·0	0·0
18·5	1·7
No. mosquitoes dissected, 58; mean M.I., 15·31.	

Table 3 shows the results of laboratory experiments in which the organic content of field breeding-water was increased by adding fresh cow-dung to the water. It will be seen that the maxillary index of population I became higher. These results indicate that it is possible for an *A. gambiae* population which would otherwise have developed as paucidentate to change into a multidentate one, by the mere fortuitous dropping of dung by cattle into the breeding place. It has been postulated[9] that a higher maxillary index confers on the mosquito a greater ability to feed on cattle and other thick-skinned domestic animals. Consequently, it has been assumed that the prevalence of multidentate mosquitoes, in an area with a high cattle population, proves that natural selection has acted in favour of such mosquitoes. However, the present observations would seem to indicate that the co-existence of multidentate mosquitoes and high cattle populations, in an area, may simply be the result of a greater proportion of the breeding places being polluted with dung and other organic matter of animal origin; which, in turn, increases the maxillary dentition of the mosquitoes.

Table 3. Maxillary Index (M.I.) of Population I Bred in 'Cow-Dunged' Field Water in the Laboratory (room temperature varied 22–25·5° C.)

Maxillary Index	Frequency (per cent)
11·5	1·2
12·0	4·9
12·5	13·6
13·0	17·3
13·5	21·0
14·0	16·0
14·5	14·8
15·0	7·4
15·5	2·5
16·0	1·2
No. mosquitoes dissected, 81; mean M.I., 13·58.	

This work was carried out under a scheme of research on tropical swamps supported by the Nuffield Foundation and undertaken by the Departments of Botany and Zoology, at Makerere University College, Kampala, Uganda.

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Rectal Glands in Woodlice

The structure and function of the rectal glands of insects have been investigated by several workers[1]. Later, Wigglesworth[2] studied these glands more thoroughly in a number of species. No report, however, has yet appeared in the literature regarding the presence of rectal glands in woodlice (Crustacea: Isopoda), although Edney[3] stated: "the fæcal pellets are moist, and there appears to be no mechanism comparable with the rectal glands of insects for reabsorption of water contained in the rectal contents". This observation, however, was not based on any experimental or histological studies.

The present report is concerned with two common species of woodlice in Great Britain, *Oniscus asellus* L. and *Porcellio scaber* Latr. Examination of the rectal contents of these animals shows that they are in a much more fluid state than the fæcal pellets, which are

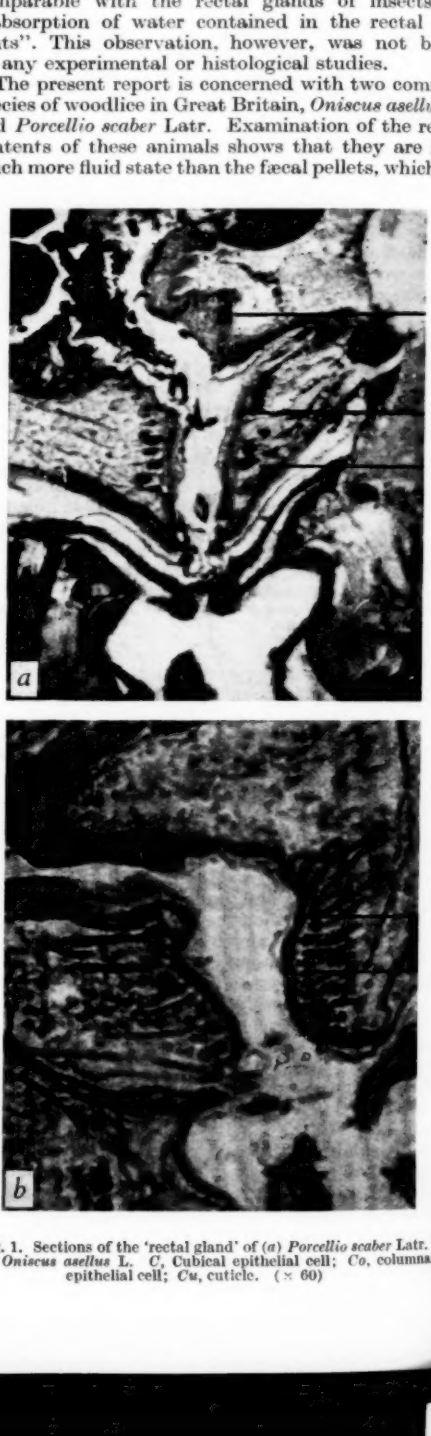

Fig. 1. Sections of the 'rectal gland' of (*a*) *Porcellio scaber* Latr.; (*b*) *Oniscus asellus* L. *C*, Cubical epithelial cell; *Co*, columnar epithelial cell; *Cu*, cuticle. (× 60)

finally discharged from the rectum in the form of rather black, large, solid, and cylindrical pellets. The water content of these pellets has been determined by Kuenen[4].

Considerable difficulty has been experienced by workers in the histological study of woodlice[5]. In the present work a variety of fixatives has been used[6], among which the best results were obtained with Carnoy's fixative. After fixing, the tissue was passed through decreasing strengths of alcohol down to water. In order to provide support, the tissue was embedded in agar and dehydrated. After double embedding, microtome sections were prepared in the usual way and the sections stained by the phloxine–tartrazine method of Lendrum[7].

In both *Oniscus asellus* and *Porcellio scaber* the lower part of the rectum is narrow, with a very thin cuticle covering cubical epithelium thrown into very small longitudinal folds (Fig. 1). In the posterior part of the rectum the cubical epithelial cells are absent and just below the cuticle lie columnar epithelial cells, tracheæ, muscle fibres and connective tissue. This structure is exactly similar to that described by Wigglesworth[2] in insects as rectal glands. These glands completely encircle the posterior part of the rectum.

Experimental evidence has been brought forward by Wigglesworth (ref. 2*a*) that rectal glands are concerned with absorption of water in insects. Water is the most important ecological factor in the habitat of woodlice, owing to the absence of cuticular wax layer. Desiccated woodlice are said to absorb water through the anus[8], and this has been confirmed in the present work. Further work is needed to elaborate the functions of the rectal glands in woodlice. Possibly these glands, too, are concerned with absorption of water from the rectal contents, although some is still lost in the fæcal pellets[4].

My thanks are due to Prof. J. L. Cloudsley-Thompson for his supervision during the work.

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Mechanism of Differential Staining of Nucleic Acids

WORK in this Department, to be published elsewhere, has shown that several histological methods for the demonstration of mucin depend for their selectivity on the use of cations of large size. Thus alcian blue, with a molecular weight of about 1,341 (ref. 1), is almost specific for acid mucins, being apparently unable to enter other, more dense, basophilic structures. The dialysed iron method[2] similarly depends on the use of large particles (colloidal ferric hydroxide), while in Southgate's mucicarmine the small carmine molecule is probably adsorbed on to colloidal aluminium hydroxide.

Arising from this work, it was thought that size of cation might influence the selectivity of other commonly used basic dyes. In the present work, fourteen basic aniline dyes have been investigated, ranging in molecular weight from 264 to 1,341 (Table 1). A variety of formalin- or Helly-fixed tissues has been used, including frog pancreas, liver, ovary, intestine, stomach and kidney, rat trachea, stomach, pancreas and intestine, and monkey pancreas.

Table 1

Dye and source	Molecular weight	Cationic weight	Percentage solubility Alcohol	Water
Alcian blue 8*GN*150 (I.C.I.)	*c.* 1,341	1,341 (?)	6·0	9·5
Victoria blue 4*R* (National Aniline Co.)	520	485	20·0	3·0
Janus green *B* (National Aniline Co.)	483	448	1·0	5·0
Bismarck brown *R* (National Aniline Co.)	461	388	3.0	1·5
Methyl green *OO* (Grübler)	458	387	3·0	8·0
Hofmann violet (Merck)	422	387	7·5	6·0
Crystal violet (National Aniline Co.)	408	373	8·75	9·0
Nile blue *A* (National Aniline Co.)	733	316	5·0	6·0
Safranin *T* (Merck)	351	316	3·5	4·5
Brillantcresylblau (Grübler)	318	282	2·0	3.0
Toluidine blue (Grübler)	306	271	1·75	3·25
Pyronin (Grübler)	302	267	0·5	9·0
Azur *A* (National Aniline Co.)	292	257	?	?
Thionin (Grübler)	264	229	1·0	1·0

(1) Molecular weights and solubilities in alcohol and water are from ref. 1. Cationic weights are calculated from data from the same source.
(2) One molecule of nile blue *A* gives rise to two cations on ionization.

In initial experiments 0·01 *M* aqueous solutions of the dyes were used, the stained, air-dried sections being mounted directly in '*D.P.X.*' (synthetic mountant, British Drug Houses). It was found that in these conditions sections were markedly overstained by certain dyes (notably victoria blue 4*R*, bismarck brown *R* and hofmann violet). It is noteworthy that most of the dyes concerned are more soluble in alcohol than in water (Table 1), and it is possible that this is a reflexion of some property common to this group of dyes, which is responsible for a loose, non-ionic bonding with tissue components. This is to some extent confirmed by the fact that thorough dehydration in ethyl alcohol (one minute each in 90 per cent, 95 per cent and absolute alcohol) was sufficient to remove the excess dye.

The results about to be described were obtained on sections stained for 15 min. in a 0·005 *N* solution of the dye in veronal-acetate buffer at *p*H 4·25, washed in tap water, dehydrated in alcohol and mounted in '*D.P.X.*'.

Under these conditions alcian blue (weight of cation about 1,341) stained nuclei minimally, and did not stain nucleoli or cytoplasmic ribonucleic acid. Victoria blue 4*R*, janus green, bismarck brown *R*, methyl green *OO*, hofmann violet and crystal violet (with cationic weights from 485 down to 373) stained nuclei more deeply than nucleoli or cytoplasmic ribonucleic acid, while safranin and nile blue (cationic weight 316) stained nuclei, nucleoli and cytoplasmic ribonucleic acid with approximately equal intensity.

XUM

Brilliant cresyl blue, toluidine blue, pyronin, azur *A* and thionin (cationic weights from 282 down to 229) stained nucleoli and cytoplasmic chromidial substance more intensely than they stained nuclei.

From these results it appears that dye cations having a combined atomic weight of between about 350 and 500 are able to penetrate into and become attached to nuclear deoxyribonucleic acid (DNA), but do not as readily stain the nucleoli and cytoplasmic granules of ribonucleic acid (RNA), which are presumably denser and better stained by smaller dye cations (of a combined atomic weight of less than about 280).

Differential staining of DNA and RNA by mixtures of two basic dyes (as in the classical methyl green/pyronin method) is now readily explicable. The proportions of the two dyes are chosen in such a way that the larger cation (methyl green) competes under favourable conditions for the DNA, while the smaller cation (pyronin) stains only RNA. If the principal factor in such differential staining is the weight of the dye cations, it should be possible to reproduce the 'methyl green/pyronin effect' (differential staining of DNA and RNA) with other pairs of basic dyes, provided that the dyes chosen are of sufficiently different cationic weights. The dyes must also, of course, be of contrasting colours, be mixed in appropriate proportions and be used at the correct *p*H.

To confirm this prediction, 1 per cent solutions of several basic dyes were prepared. Paraffin sections were stained for 15 min. in a mixture of a pair of such types (combined in various proportions), rinsed in tap water, dehydrated thoroughly in alcohol and mounted in '*D.P.X.*'. The following dye pairs stained DNA and RNA differentially under these conditions, when mixed in correct proportions: bismarck brown *R*/toluidine blue, bismarck brown *R*/azur *A*, victoria blue 4*R*/hofmann violet, victoria blue 4*R*/safranin, victoria blue 4*R*/pyronin, janus green/pyronin and janus green/safranin. In every case, the dye with the larger cation stained the nuclei, while the smaller cation stained nucleoli and cytoplasmic chromidial substance. A few nuclei, especially the small nuclei of frog red blood cells, stained with the smaller cation instead of the larger. This may be due to such nuclei lying wholly within the plane of the section; in such a case the nuclear membrane will be intact, and prevent access of the larger cation[3].

Dye pairs in which there was a large difference between the weights of the cations gave particularly good discrimination between DNA and RNA. Thus victoria blue 4*R*/pyronin or janus green/pyronin (mixed in a proportion of one part by weight of the larger cation to four parts of the smaller) gave results which compared favourably with those obtainable with the classical methyl green/pyronin method. Where the difference between the cationic weights of the two dyes was not large (for example, victoria blue 4*R*/hofmann violet), or where incorrect proportions of the two dyes were used, good differential staining of nuclei and nucleoli was sometimes obtained, but cytoplasmic RNA stained with both dyes. This is taken to indicate that the density of cytoplasmic RNA granules is intermediate between that of DNA and that of the nucleolus.

The use of basic dyes of varying cationic weights can also give some indication of the change in density of a structure under different conditions. Thus the staining of so-called 'depolymerized' DNA with pyronin instead of methyl green in the methyl green/pyronin method[4] probably indicates that the heat, or other treatment to which the DNA has been subjected, has altered its structure in such a way as to increase its density, and render access of the larger cation more difficult.

Dye-pairs in which there was relatively little difference between the cationic weights (for example, crystal violet/pyronin or safranin/thionin) gave little or no differential staining of DNA and RNA, irrespective of the proportions in which the dyes were mixed.

It appears from these results that the chemical explanations which have been advanced to explain the specificity of the methyl green/pyronin method[4] are unnecessary, the chief factor being the weights of the dye cations. The same factor explains the effect of the dye mixtures, malachite green/acridine red, methyl green/toluidine blue and celestine blue/pyronin, which give the same empirical results as methyl green/pyronin[4]; malachite green has a cationic weight of 328, celestine blue 329 and acridine red 240.

It should not be concluded from the above that the weight of the cation is the only relevant factor in staining with basic dyes. Mordants, *p*H, solubilities in various media, etc., may also play an important part in some procedures[5].

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Dispersal of Tropical Seeds by Ocean Currents

DURING the winter of 1956 and the following summer, farmers living near the Ninety Mile Beach on the west coast of North Auckland (latitude 34–35° S.) noticed that numbers of unknown fruits and seeds, recognized as of tropical origin, were being washed ashore. Specimens were forwarded to the Botany Division, Department of Scientific and Industrial Research, by Mr. H. G. Halliwell, instructor in agriculture, Kaitaia, for identification.

The fruits and seeds were all of kinds known to be carried long distances by ocean currents[1]. They were: fruit of *Barringtonia asiatica*, fruits of a cycad, seeds of *Entada* sp. (nicker bean), *Guilandina crista* (Moluccs bean or grey nicker), *Mucuna gigantea*, seeds apparently of two other species of *Mucuna*, and nuts of *Aleurites fordii* (candlenut). There was also a red leguminous seed 17 mm. long and an unidentified fruit, possibly from one of the Myristicaceae. Apparently nicker beans and candlenuts were the most common. According to a newspaper report[2], at times many coconuts were being washed up. Candlenuts from Mangonui, on the east coast at about the same latitude, were also sent in by Dr. A. Couper, of the Geological Survey. They were called in the north 'petrified walnuts'. Some of the material must have been afloat for a considerable time, for the *Barringtonia* fruit, most of the cycad fruits, and many

of the nicker beans had remains of a polyzoan on them, and were sometimes quite covered.

Although the number and variety seem to be unusual, this is not the first time that tropical seeds have drifted to New Zealand. It was known in Auckland thirty or so years ago that nicker beans were cast up on the coasts of North Auckland from time to time. The newspaper report refers to present conditions as "a reversion to an earlier pattern of ocean currents" and says "in greater profusion than in past years all sorts of tropical flotsam are being cast up".

Seven out of twenty-two seeds of *Entada* sown at Lincoln, Christchurch, in November 1956, germinated about 21 months later. Two of the plants are now growing in the glasshouses, one a vine 4 ft. long, the other 8 ft. Another plant in the tropical house, Botanic Gardens, Christchurch, is 12 ft. long. In North Auckland plants have been grown successfully outdoors by local farmers, and one plant is reported to have grown to a great height. As *Entada* sp. in Fiji is most characteristically found in the mangrove formation and also grows in the forest[1], it seems unlikely that it will become established of its own accord on the Ninety Mile Beach, an exposed shore backed by sand-dune country.

The candlenuts were either empty or the seed had decayed into a soft oily mass, as reported previously for seeds of this species carried by ocean currents.

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Classification of the Tea Plant

Dr. W. Wight, referring to Watt and Mann's categories of leaf-form within the Assam variety of tea plant[1], goes on to say: "Number of leaf veins was emphasized; but this has not proved to be a satisfactory criterion". I may say at once that Watt was solely responsible for the leaf vein idea for separating the varieties of tea. When I wrote, with him, the second edition of the *Pests and Blights of the Tea Plant* I had to include the leaf vein idea, but I never was, myself, able to make anything out of it. I never agreed with Watt on this subject, and it was included in our book in deference to him. Thus, so far as I am concerned, the number of leaf veins was an unsatisfactory criterion fifty-seven years ago.

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Bivia, Woodside,
Aspley Guise,
Bletchley.

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Staining Reaction of the Pit Membrane of Wood Cells

In a recent paper Harada and Wardrop[1] report that the lignin content of the ray parenchyma cell walls of *Cryptomeria japonica* (Linnæus fil.) Don. is about 43·5 per cent and also that the lignin is distributed nearly uniformly through the cell walls. In view of the bearing on their observations and also the possible relation to the movement of materials through wood, the results of the reaction of the pit membrane when wood sections are treated with differential stains are recorded.

Radial longitudinal sections of approximately 10μ were stained with safranin and fast green according to the following schedule: (1) Sections warmed with aqueous safranin for 3–5 min.; (2) washed with several changes of water and then dehydrated in the normal way with ethyl alcohol; (3) stained with fast green in clove oil for approximately 15 sec. (this time can be increased with dilute fast green); (4) washed with clove oil, cleared with xylene and mounted in canada balsam. Washing was continued until the wash liquid was colourless.

In the following species the pit membranes of the ray parenchyma to tracheid pits were stained green in contrast to the red stained unpitted wall:

Pinus radiata D. Don, *P. khaysa* Royle, *Pseudotsuga menziesii* (Mirb.) Franco, *C. japonica*, *Callitris hugelii* (Carr) Franco, *Abies lasiocarpa* (Hooker) Nuttall. The pit membranes of the ray tracheid to tracheid pits were not differentiated to the same degree as the ray parenchyma pit membrane. Both in *P. radiata* and *C. hugelii* the torus of the bordered pits of the tracheids was stained green.

With the following species the membranes of the ray parenchyma to vessel pits and the parenchyma pits generally were differentiated green in contrast to the unpitted cell wall, which was stained red:

Sloanea woollsii F. Muell., *Elaeocarpus grandis* F. Muell., *Cryptocarya glaucescens* R. Br., *Flindersia schottiana* F. Muell., *Toona australis* Harms, *Sterculia acerifolia* A. Cunn., *Eucalyptus viminalis* Labill., *E. pilularis* Sm., *E. microcorys* F. Muell. Due to their small size the pit membranes of the fibres were hard to detect, but in a number of the above species a distinct green coloration was obtained. Of the species examined, only *Nothofagus moorei* J. H. Maiden failed to show differentiation of the pit membrane of the ray-vessel pits. The pit membrane in this species appeared to be masked by abundant polyphenolic materials.

Where possible, sapwood rather than heartwood was used for these observations as it was found that the polyphenolic materials deposited during the transition from sapwood to heartwood obscured the pit membrane, showing instead a red stain with the safranin.

According to Johansen[2], safranin and fast green adequately differentiate lignified and non-lignified tissue, so that this differentiation indicates that the pit membranes in a number of cells are not lignified. This observation is interesting, as it is those layers which make up the pit membrane, namely, the primary wall and middle lamella, which have the highest concentration of lignin in the unpitted cell wall[3]. The pit membrane, therefore, retains the unlignified condition of the newly differentiated cell wall and explains how the remarkable expansion of the pit membrane which occurs during tyloses is possible. The tylosed pit membrane in *Angophora costata* Domin. and *E. microcorys* on the basis of the above staining reaction did not appear to be lignified.

While Liese[4] and Harada *et al.*[5] describe encrusting material present in the pit membrane, it would appear that this material is not lignin. The absence of lignin in the pit membrane has been indicated by means of other techniques by A. B. Wardrop (private communication). The fact that lignin is confined to those parts of the wall where a secondary wall is

formed implies very selective deposition, as mentioned by Wardrop and Bland[3]. The foregoing staining reaction serves also as an excellent technique for clearly defining the size and shape of the pit membrane and it has been suggested that this technique should have general application in anatomical work.

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A Sealing Compound for use in Biological Work

In the course of rhizosphere studies, it was desired to measure continuously the respiration of intact sterile roots during the growth and development of a variety of plants. To separate the photosynthetic and respiratory activities of the portions above ground from the respiration of the roots, a material was required to prevent diffusion of gases from one zone to the other. The material had to fulfil several requirements in order to be effective: (1) be impermeable to gases and water; (2) be non-toxic to plants; (3) cause no physical damage to plants; (4) be sufficiently pliable to permit normal enlargement of stems; (5) withstand a continuous positive pressure of 0·5–1·0 lb./in.²; (6) resist microbial and environmental deterioration; (7) be autoclavable; (8) be easily handled and applied; (9) be relatively inexpensive.

Although a variety of substances was tested, only one compound has been found which fulfilled all these requirements. This material is a viscous silicone fluid which solidifies at room temperatures on the addition of a catalyst. (Material is marketed by the Dow Corning Corporation, Midland, Michigan, under the trade name of 'Silastic *RTV*' (room temperature vulcanizing silicone rubber), and by the General Electric Co., Silicone Products Department, Waterford, New York, under the name of 'Silicone Rubber *RTV*'.) The time for complete vulcanization can be controlled by the type and amount of catalyst used; working time can be adjusted from 10 min. to several days. The plasticity can be increased by the addition of a silicone thinner (Dow Corning '200 Fluid', 20 centistokes). The base, catalyst, and thinner may be autoclaved separately and mixed aseptically just prior to pouring around the stem.

The rubber-like solid forms an impermeable seal with glass, metal, certain plastics, sand, and with plant roots and stems. Although a continuous positive pressure of only 0·5–1·0 lb./in.² is regularly used in our work, the material is capable of withstanding considerably higher pressures, both positive and negative. A layer 10 mm. thick and 38 mm. in diameter withstood a continuous positive pressure of 7 lb./in.² and a continuous negative pressure of 500 mm. mercury; a layer 10 mm. thick and 70 mm. in diameter withstood a pressure of 2 lb./in.². At higher pressures, the sealer itself does not leak, but the interface bond between the sealer and either the wall of the glass tube or the plant stem is ruptured.

Although a gas-tight seal is formed around the stem of plants, the seal is sufficiently pliable to permit normal development of the stem as well as of the entire plant. The consistency of the vulcanized product does not change during autoclaving nor during prolonged use, and may be employed at temperatures ranging from −70 to 500° F. Other physical and chemical properties are described by the manufacturers.

In our work, surface sterilized seeds are planted in short glass growth tubes (38 mm. internal diameter) contained in specially designed culture units, which will be described fully elsewhere. The roots grow into a sterile respiration chamber, and the upper portions of the plant are allowed to develop in air. To separate the two zones, the sealer is poured around the plant stem inside the growth tubes and allowed to solidify before a continuous air-stream is passed through the root respiration chamber. Both monocotyledonous and dicotyledonous plants have been successfully cultured in the presence of this material: legumes and corn have produced fruit; cotton, crucifers, cucurbits, and seeded bananas are at present growing vigorously.

Other compounds tested as sealers were not suitable for several reasons. Various combinations of paraffin, petrolatum and lanolin, as well as some carboxyvinyl polymers, were extruded from the growth tubes when pressure was applied; this difficulty was accentuated when the temperature of the environment was high. Several materials, for example, latex, poly*iso*butylenes, urethane polymers, polyacrylic and polyvinyl resins, were toxic; some set too rapidly for easy handling; some, for example, urethane polymers and some microcrystalline waxes, cured to a consistency that constricted the stem and prevented normal development; some, for example, various paraffin-petrolatum mixtures, butyl-latex emulsions, distilled acetylated monoglycerides, carboxyvinyl polymers, shrank on curing and failed to form a gas-tight interface with either the glass tube or the plant stem; some did not withstand autoclaving; and others, such as the polyethylene glycols, being water-soluble, were dissolved by condensation, transpiration and guttation water.

In addition to being used in sterile root cultures, the silicone material is also being employed in these Laboratories to seal surface-sterilized intact roots in tubes containing root-infecting organisms (Halmos, S., and Beckman, C. H., unpublished results); to make gas-tight gaskets for attaching respiration and transpiration chambers to leaves[1]; to make impressions of leaf surfaces for microscopic examination (Brun, W. A., unpublished results; and Zelitch, I., unpublished results); and to make closures for odd-shaped and hard-to-reach openings which pre-manufactured rubber stoppers do not fit. Additional applications are described by the manufacturers.

The non-toxicity of this compound to plants, as well as its other properties, should make it useful in a variety of biological studies.

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MICROBIOLOGY

Activation of Amino-Acids in Micro-Organisms which produce Antibiotics

It seems well established that the first steps in the biosynthesis of proteins are the activation of amino-acids in the presence of adenosine triphosphate (ATP) and the transfer of the activated amino-acids to a soluble fraction of ribonucleic acid (RNA)[1]. Such reactions are also involved in the biosynthesis of some peptides, for example, the condensation of glutamyl-cysteine with glycine to give glutathione[2]. Enzymes activating almost all the naturally occurring L-amino-acids have been isolated from animal, plant, and microbial sources. Only one enzyme active on a D-amino acid, D-alanine, has been demonstrated in cells of *Lactobacillus arabinosus* and other Lactobacillaceae[3].

If the mechanisms for protein synthesis are involved also in the synthesis of peptides, it should be possible to demonstrate activation and transfer of amino-acids present in peptides but absent from proteins, such as many of the D-amino-acids. To test this hypothesis the following micro-organisms were selected, since they produce antibiotics containing one or more of the amino-acids that do not occur in proteins: *Penicillium chrysogenum P–4* (Lepetit S.p.A.), producing penicillin that contains D-valine; *Streptomyces antibioticus* 23 (Lepetit S.p.A.) and *Streptomyces parvus N.R.R.L. B-1455* (both producing antibiotics of the actinomycin group in which D-valine, sarcosine, D-allo*iso*leucine and N-methyl-L-valine are present); *Bacillus polymyxa A.T.C.C.* 10401 and *B. polymyxa* 2459 (Ch. Pfizer and Co.) producing, respectively, polymyxin-D and polymyxin-B, containing D-phenylalanine, D-leucine, D-serine, D- and L-α,γ-diaminobutyric acid; *Bacillus circulans A.T.C.C.* 14040, producing circulin containing D-leucine and L-α,γ-diaminobutyric acid. The micro-organisms were grown on complex media capable of supporting antibiotic synthesis and were collected when the production of the antibiotic reached its maximum (2 days for the bacteria, 3 for *P. chrysogenum*, and 6 to 8 for the Streptomycetes). Cell-free extracts were prepared by suspending the centrifuged cells in one volume of 0·066 *M* phosphate buffer and 0·002 *M* reduced glutathione (*p*H 6·9) and disrupting them with glass beads in a refrigerated mechanical disintegrator. Following centrifugation at 20,000*g* for 20 min., the presence of amino-acid-activating enzymes was assayed on the supernatants by measuring the exchange between ATP and radioactive pyrophosphate[4].

The results (Table 1) indicate that, while a fair number of the L-amino-acids is activated, no activation of the D-amino-acids is detectable even if the micro-organism is capable of synthesizing compounds containing amino-acids with such an optical configuration. In addition, sarcosine, which has no optical activity, is not activated by the tested Streptomycetes. L-α,γ-diaminobutyric acid, which has not been found in proteins, is activated by strain 2459 of *B. polymyxa*; this is the first time that this amino-acid is reported to be activated.

The possibility that the enzymes activating the D-amino-acids and sarcosine through an ATP-pyrophosphate exchange are destroyed during the process of extraction or are present in too small amounts for detection cannot be ruled out, but seems unlikely. It appears more probable that the pathway of biosynthesis of these peptides does not involve the activation of the D-amino-acids. Such a hypothesis is in agreement with the recent findings that exclude a process of amino-acid activation in the biosynthesis of some petides containing D-alanine and other L-amino-acids by *Staphylococcus aureus*[5]. Work under way in this laboratory[6] has shown that while D-leucine-^{14}C is incorporated in the polymyxin-D synthesized by *B. polymyxa*, the amino-acid is not activated by an ATP-pyrophosphate exchange nor transferred to soluble RNA.

Table 1. ACTIVATION OF AMINO-ACIDS
Micromoles of pyrophosphate-phosphorus-32 exchanged per 15 min. per mgm. of protein

	B. polymyxa 10401	*B. polymyxa* 2459	*B. circulans*	*Str. antibioticus*	*Str. parvus*	*P. chrysogenum*
L-Alanine	0	0	0·077	0	0	0·012
L-Arginine	0·013	0	0	0·115	0·044	0
L-Aspartic acid	0·080	0	0	0	0	0
L-Cysteine	0·243	—	—	0·107	0·059	0
L-α,γ-Diaminobutyric acid	0	0·641	0	—	—	—
L-Glutamic acid	0	0	0	0·062	0	0
Glycine	0	0	0	0·133	0·075	0
L-Histidine	0	0·182	0	0·986	0	0
L-*iso*Leucine	2·001	0·695	0·490	1·129	0·162	0·116
L-Leucine	2·073	1·690	3·623	0·498	0·044	0·124
L-Lysine	0·094	0·140	0	0·098	0	0·020
L-Methionine	0·278	0·416	0·487	0·444	0	0
L-Phenylalanine	0·064	0	0	0	0	0·016
L-Proline	0·144	0	0	0·204	0	0
L-Serine	0	0·055	0	0·302	0	0·039
L-Threonine	0·210	0·050	0·035	0·088	0·044	0
L-Tryptophan	0·083	0	—	0·133	0	0
L-Valine	0·106	1·790	0·986	0·489	0·235	0·076
Sarcosine	—	—	—	0	0	—
D-Alanine	0	0·162	0	0	0	0
D-*iso*Leucine	0	0	—	—	—	—
D-Leucine	0	0	0	0	0	0
D-Phenylalanine	0	0	0	—	—	0
D-Serine	0	0	0	—	—	—
D-Threonine	0	0	0	—	—	—
D-Valine	—	—	—	0	0	0
None (control)	0·133	0·280	0·355	0·507	0·073	0·100

The assay system contained 20 μM of *tris* buffer (*p*H 7·9); 5 μM of ATP (*p*H 6·5); 2·5 μM of $MgCl_2$; 3·2 μM of sodium pyrophosphate-phosphorus-32 (corresponding to approximately 340,000 counts per min. on a 'Tracerlab' mica window counter); 10 μM of amino-acid; cell-free extracts ranging from 0·068 to 0·49 mgm. of protein; glass distilled water, 0·5 ml.

The values obtained in the controls containing no amino-acids were subtracted from all the experiments. The activation was considered present when the experimental data were at least 10 per cent higher than the controls.

We wish to acknowledge the kind release of cultures from Lepetit S.p.A., Milan, and Charles Pfizer and Co., Brooklyn, N.Y. This work has been supported by a grant of the International Institute for Scientific Research.

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formed implies very selective deposition, as mentioned by Wardrop and Bland[3]. The foregoing staining reaction serves also as an excellent technique for clearly defining the size and shape of the pit membrane and it has been suggested that this technique should have general application in anatomical work.

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In the course of rhizosphere studies, it was desired to measure continuously the respiration of intact sterile roots during the growth and development of a variety of plants. To separate the photosynthetic and respiratory activities of the portions above ground from the respiration of the roots, a material was required to prevent diffusion of gases from one zone to the other. The material had to fulfil several requirements in order to be effective: (1) be impermeable to gases and water; (2) be non-toxic to plants; (3) cause no physical damage to plants; (4) be sufficiently pliable to permit normal enlargement of stems; (5) withstand a continuous positive pressure of 0·5–1·0 lb./in.^{2}; (6) resist microbial and environmental deterioration; (7) be autoclavable; (8) be easily handled and applied; (9) be relatively inexpensive.

Although a variety of substances was tested, only one compound has been found which fulfilled all these requirements. This material is a viscous silicone fluid which solidifies at room temperatures on the addition of a catalyst. (Material is marketed by the Dow Corning Corporation, Midland, Michigan, under the trade name of 'Silastic *RTV*' (room temperature vulcanizing silicone rubber), and by the General Electric Co., Silicone Products Department, Waterford, New York, under the name of 'Silicone Rubber *RTV*'.) The time for complete vulcanization can be controlled by the type and amount of catalyst used; working time can be adjusted from 10 min. to several days. The plasticity can be increased by the addition of a silicone thinner (Dow Corning '200 Fluid', 20 centistokes). The base, catalyst, and thinner may be autoclaved separately and mixed aseptically just prior to pouring around the stem.

The rubber-like solid forms an impermeable seal with glass, metal, certain plastics, sand, and with plant roots and stems. Although a continuous positive pressure of only 0·5–1·0 lb./in.^{2} is regularly used in our work, the material is capable of withstanding considerably higher pressures, both positive and negative. A layer 10 mm. thick and 38 mm. in diameter withstood a continuous positive pressure of 7 lb./in.^{2} and a continuous negative pressure of 500 mm. mercury; a layer 10 mm. thick and 70 mm. in diameter withstood a pressure of 2 lb./in.^{2}. At higher pressures, the sealer itself does not leak, but the interface bond between the sealer and either the wall of the glass tube or the plant stem is ruptured.

Although a gas-tight seal is formed around the stem of plants, the seal is sufficiently pliable to permit normal development of the stem as well as of the entire plant. The consistency of the vulcanized product does not change during autoclaving nor during prolonged use, and may be employed at temperatures ranging from −70 to 500° F. Other physical and chemical properties are described by the manufacturers.

In our work, surface sterilized seeds are planted in short glass growth tubes (38 mm. internal diameter) contained in specially designed culture units, which will be described fully elsewhere. The roots grow into a sterile respiration chamber, and the upper portions of the plant are allowed to develop in air. To separate the two zones, the sealer is poured around the plant stem inside the growth tubes and allowed to solidify before a continuous air-stream is passed through the root respiration chamber. Both monocotyledonous and dicotyledonous plants have been successfully cultured in the presence of this material: legumes and corn have produced fruit; cotton, crucifers, cucurbits, and seeded bananas are at present growing vigorously.

Other compounds tested as sealers were not suitable for several reasons. Various combinations of paraffin, petrolatum and lanolin, as well as some carboxyvinyl polymers, were extruded from the growth tubes when pressure was applied; this difficulty was accentuated when the temperature of the environment was high. Several materials, for example, latex, poly*iso*butylenes, urethane polymers, polyacrylic and polyvinyl resins, were toxic; some set too rapidly for easy handling; some, for example, urethane polymers and some microcrystalline waxes, cured to a consistency that constricted the stem and prevented normal development; some, for example, various paraffin-petrolatum mixtures, butyl-latex emulsions, distilled acetylated monoglycerides, carboxyvinyl polymers, shrank on curing and failed to form a gas-tight interface with either the glass tube or the plant stem; some did not withstand autoclaving; and others, such as the polyethylene glycols, being water-soluble, were dissolved by condensation, transpiration and guttation water.

In addition to being used in sterile root cultures, the silicone material is also being employed in these Laboratories to seal surface-sterilized intact roots in tubes containing root-infecting organisms (Halmos, S., and Beckman, C. H., unpublished results); to make gas-tight gaskets for attaching respiration and transpiration chambers to leaves[1]; to make impressions of leaf surfaces for microscopic examination (Brun, W. A., unpublished results; and Zelitch, I., unpublished results); and to make closures for odd-shaped and hard-to-reach openings which pre-manufactured rubber stoppers do not fit. Additional applications are described by the manufacturers.

The non-toxicity of this compound to plants, as well as its other properties, should make it useful in a variety of biological studies.

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MICROBIOLOGY

Activation of Amino-Acids in Micro-Organisms which produce Antibiotics

It seems well established that the first steps in the biosynthesis of proteins are the activation of amino-acids in the presence of adenosine triphosphate (ATP) and the transfer of the activated amino-acids to a soluble fraction of ribonucleic acid (RNA)[1]. Such reactions are also involved in the biosynthesis of some peptides, for example, the condensation of glutamyl-cysteine with glycine to give glutathione[2]. Enzymes activating almost all the naturally occurring L-amino-acids have been isolated from animal, plant, and microbial sources. Only one enzyme active on a D-amino acid, D-alanine, has been demonstrated in cells of *Lactobacillus arabinosus* and other Lactobacillaceae[3].

If the mechanisms for protein synthesis are involved also in the synthesis of peptides, it should be possible to demonstrate activation and transfer of amino-acids present in peptides but absent from proteins, such as many of the D-amino-acids. To test this hypothesis the following micro-organisms were selected, since they produce antibiotics containing one or more of the amino-acids that do not occur in proteins: *Penicillium chrysogenum P*–4 (Lepetit S.p.A.), producing penicillin that contains D-valine; *Streptomyces antibioticus* 23 (Lepetit S.p.A.) and *Streptomyces parvus N.R.R.L. B*-1455 (both producing antibiotics of the actinomycin group in which D-valine, sarcosine, D-allo*iso*leucine and N-methyl-L-valine are present); *Bacillus polymyxa A.T.C.C.* 10401 and *B. polymyxa* 2459 (Ch. Pfizer and Co.) producing, respectively, polymyxin-D and polymyxin-B, containing D-phenylalanine, D-leucine, D-serine, D- and L-α,γ-diaminobutyric acid; *Bacillus circulans A.T.C.C.* 14040, producing circulin containing D-leucine and L-α,γ-diaminobutyric acid. The micro-organisms were grown on complex media capable of supporting antibiotic synthesis and were collected when the production of the antibiotic reached its maximum (2 days for the bacteria, 3 for *P. chrysogenum*, and 6 to 8 for the Streptomycetes). Cell-free extracts were prepared by suspending the centrifuged cells in one volume of 0·066 *M* phosphate buffer and 0·002 *M* reduced glutathione (*p*H 6·9) and disrupting them with glass beads in a refrigerated mechanical disintegrator. Following centrifugation at 20,000*g* for 20 min., the presence of amino-acid-activating enzymes was assayed on the supernatants by measuring the exchange between ATP and radioactive pyrophosphate[4].

The results (Table 1) indicate that, while a fair number of the L-amino-acids is activated, no activation of the D-amino-acids is detectable even if the micro-organism is capable of synthesizing compounds containing amino-acids with such an optical configuration. In addition, sarcosine, which has no optical activity, is not activated by the tested Streptomycetes. L-α,γ-diaminobutyric acid, which has not been found in proteins, is activated by strain 2459 of *B. polymyxa*; this is the first time that this amino-acid is reported to be activated.

The possibility that the enzymes activating the D-amino-acids and sarcosine through an ATP-pyrophosphate exchange are destroyed during the process of extraction or are present in too small amounts for detection cannot be ruled out, but seems unlikely. It appears more probable that the pathway of biosynthesis of these peptides does not involve the activation of the D-amino-acids. Such a hypothesis is in agreement with the recent findings that exclude a process of amino-acid activation in the biosynthesis of some petides containing D-alanine and other L-amino-acids by *Staphylococcus aureus*[5]. Work under way in this laboratory[6] has shown that while D-leucine-^{14}C is incorporated in the polymyxin-D synthesized by *B. polymyxa*, the amino-acid is not activated by an ATP-pyrophosphate exchange nor transferred to soluble RNA.

Table 1. Activation of Amino-Acids
Micromoles of pyrophosphate-phosphorus-32 exchanged per 15 min. per mgm. of protein

	B. polymyxa 10401	*B. polymyxa* 2459	*B. circulans*	*Str. antibioticus*	*Str. parvus*	*P. chrysogenum*
L-Alanine	0	0	0·077	0	0	0·012
L-Arginine	0·013	0	0	0·115	0·044	0
L-Aspartic acid	0·080	0	0	0	0	0
L-Cysteine	0·243	—	—	0·107	0·059	0
L-α,γ-Diaminobutyric acid	0	0·641	0	—	—	—
L-Glutamic acid	0	0	0	0·062	0	0
Glycine	0	0	0	0·133	0·075	0
L-Histidine	0	0·182	0	0·986	0	0
L-*iso*Leucine	2·001	0·695	0·490	1·129	0·162	0·116
L-Leucine	2·073	1·690	3·623	0·498	0·044	0·124
L-Lysine	0·094	0·140	0	0·098	0	0·020
L-Methionine	0·278	0·416	0·487	0·444	0	0
L-Phenylalanine	0·064	0	0	0	0	0·016
L-Proline	0·144	0	0	0·204	0	0
L-Serine	0	0·055	0	0·302	0	0·039
L-Threonine	0·210	0·050	0·035	0·088	0·044	0
L-Tryptophan	0·083	0	—	0·133	0	0
L-Valine	0·106	1·790	0·986	0·489	0·235	0·076
Sarcosine	—	—	—	0	0	—
D-Alanine	0	0·162	0	0	0	0
D-*iso*Leucine	0	0	—	—	—	—
D-Leucine	0	0	0	0	0	0
D-Phenylalanine	0	0	0	—	—	0
D-Serine	0	0	0	—	—	—
D-Threonine	0	0	0	—	—	—
D-Valine	—	—	—	0	0	0
None (control)	0·133	0·280	0·355	0·507	0·073	0·100

The assay system contained 20 μM of *tris* buffer (*p*H 7·9); 5 μM of ATP (*p*H 6·5); 2·5 μM of $MgCl_2$; 3·2 μM of sodium pyrophosphate-phosphorus-32 (corresponding to approximately 340,000 counts per min. on a 'Tracerlab' mica window counter); 10 μM of amino-acid; cell-free extracts ranging from 0·068 to 0·49 mgm. of protein; glass distilled water, 0·5 ml.

The values obtained in the controls containing no amino-acids were subtracted from all the experiments. The activation was considered present when the experimental data were at least 10 per cent higher than the controls.

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Enzymic Binding of Hydroxylamine by Fumaric Acid

An organism similar to *E. coli* isolated in our laboratory was found to grow in a minimal medium containing glucose and ammonium sulphate in the presence of relatively large concentrations of hydroxylamine (up to 100 μgm./ml. of the hydrochloride —amounts which were highly toxic to other organisms). The amine, which was determined by the method of Csaky[1], was found to disappear during growth.

The metabolic conversion of hydroxylamine by the organism was measured using washed cell suspensions, lyophilized cells, or cell-free preparations purified by fractionation with ammonium sulphate and further treatment on a *DEAE*-cellulose column. The reaction mixture (for composition see Table 1) was incubated at a low *p*H of 5·6–5·8 in order to minimize spontaneous decomposition of the hydroxylamine.

When a sonicated cell-free extract of washed lyophilized cells was employed instead of living cells, very little, if any, hydroxylamine disappeared. The activity of the extract could, however, be restored by the addition of the wash fluid of the freshly lyophilized preparation, fumaric or malic acid. While succinate showed only slight activity, maleate and malonate were inactive. Some of the results are shown in Table 1. The rate of disappearance of hydroxylamine was dependent on the amount of fumaric or malic acid present. In fact, for each μmole of added fumarate (or malate) about 0·8–0·9 μmole of hydroxylamine was consumed[2]. Changes in the amounts of fumaric and malic acids were followed chromatographically[3].

Using aged lyophilized preparations (stored in the deep freeze at −18° C.) disappearance of hydroxylamine could be achieved by the addition of fumaric but not malic acid. Thus, fumaric acid seems to be the active substrate, while the effectiveness of malic acid can be ascribed to the presence of a labile fumarase. This assumption was confirmed by adding citrate or phthalate buffer (50 μmoles–*p*H 5·8)[4] to the reaction mixture. Under these conditions only fumaric acid was active.

Ammonia, when added instead of hydroxylamine, did not react with fumaric acid (ammonia did not disappear), thus no aspartase seems to be involved. The presence of hydroxylamine reductase[5,6] can be ruled out as no ammonia, or very little, was formed. Nitrate, nitrite or gaseous nitrogen compounds were not found.

The reaction carried out with the purified enzyme was not activated by any of the following ions: Mn^{2+}, Mg^{2+}, Co^{2+}, Zn^{2+}, Fe^{2+}, PO_4^{3-}, or adenosine triphosphate (ATP). Diphosphopyridine nucleotide (DPN) (0·3 μmole) activated some purified enzyme preparations but not others. Sodium cyanide (10^{-2} *M*) or hydrazine (10^{-2} *M*) had no inhibiting effect on the reaction.

Identification of the enzymic condensation product of hydroxylamine with fumaric acid was attempted. At the end of the reaction (after 1–2 hr. at 37° C.) the complete system and the respective controls were subjected to hydrogenation in the Parr apparatus as follows: a minute amount of platinum oxide was added to 1 ml. of the reaction mixture acidified with hydrochloric acid to about *p*H 1·0 and shaken for 6 hr. under a pressure of 15 lb./in.^{2}. The hydrogenated samples were filtered and chromatographed in order to detect amino-acids. The complete system showed a spot of aspartic acid in contrast to the various controls (without enzyme, fumaric acid or hydroxylamine). The amount of aspartic acid formed by the catalytic hydrogenation was roughly comparable to the amount of hydroxylamine which disappeared. The formation of aspartic acid when most of the hydroxylamine and fumaric acid had disappeared concomitantly suggests that hydroxylamine as such combined with fumaric acid at one of the carbon atoms adjacent to the double bond. The nature of this compound, which seems a likely intermediate, had not as yet been established. Still to be demonstrated is whether or not this compound can be enzymically reduced to aspartic acid by extracts of this micro-organism.

A varying portion of the hydroxylamine which disappeared during the reaction (from insignificant amounts to about 50 per cent) could be recovered by boiling the reaction mixture for 10 min. with 6 *N* hydrochloric acid, and the hydroxylamine determined after neutralization. Thus, the formation of a bound hydroxylamine product, as found by Endres[7] in *Azotobacter* cultures, can be shown with our *E. coli* preparation. The variability in the extent of the hydrolysis may perhaps indicate the presence, in some preparations, of an additional intermediary product and labile enzyme.

Although the enzymic reaction was studied in detail only with organisms similar to *E. coli*, we have

Table 1. Effect of Dicarboxylic Acids on the Disappearance of Hydroxylamine

		1	2	3	4	5	6	7	8
1.	Lyophilized cells (from hydroxylamine-containing medium), 100 mgm.	+							
2.	Ditto, washed twice by centrifugation		+	+	+	+	+	+	
3.	Supernatant from No. 2 concentrated to original volume (wash-fluid)			+					
4.	Sonicated, cell-free extract, 15 mgm.								+
5.	Fumaric acid, 10 μ*M*				+				+
6.	L-Malic acid, 10 μ*M*					+			
7.	Maleic acid, 10 μ*M*						+		
8.	Malonic acid, 10 μ*M*							+	
9.	Succinic acid, 10 μ*M*								
10.	Hydroxylamine, 5 μ*M*	+	+	+	+	+	+	+	+
		Optical density at 530 mμ							
Time (min.)	0	0·420	0·400	0·400	0·415	0·430	0·420	0·400	0·415
	30	0·230	0·385	0·140		0·120			
	60	0·095	0·360	0·030	0·010	0·030	0·395	0·370	0·140

The system contained in addition: pyridoxal phosphate, 5 μgm.; sodium molybdate, 5 μ*M*. Final volume, 2·5 ml.; *p*H 5·6. The reaction mixture was incubated at 37° C. and at intervals aliquots were taken and the remaining hydroxylamine determined by the method of Csaky (ref. 1), using a Coleman Junior spectrophotometer.

noted the disappearance of hydroxylamine in the presence of fumaric acid using preparations of other organisms such as *Escherichia coli B* and *Azotobacter vinelandii*.

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Induced Increase in Resistance of *Entamoeba histolytica* to Fumagillin

ALTHOUGH there has been much work on *in vitro* effects of various amœbicidal agents on *Entamoeba histolytica*, I have not found any published data dealing with the induced increase in resistance of this protozoan to amœbicidal agents. The antiamœbic activity of fumagillin was first studied by McCowen *et al.*[1] and was confirmed by Hrenoff and Nakamura[2]. Many workers have since studied the clinical efficacy of fumagillin in amœbiasis[3–7]. The present work was undertaken to determine the activity of fumagillin against a number of newly isolated strains of *E. histolytica* from a variety of clinical infections. Also, an attempt was made to increase resistance of these strains to this highly active amœbacidal antibiotic. The main reason for using fumagillin in these experiments is that it is not antibacterial in activity and therefore this agent can be studied in cultures of amœbæ growing in association with a mixed bacterial flora. The associated bacteria are required because *E. histolytica* cannot be cultivated axenically consistently. Furthermore, use of fumagillin minimizes the indirect activity of the drug on amœbæ since the bacterial flora apparently are unaffected, and the inhibition of the amœbæ can be considered as directly due to the antibiotic.

Six strains of *E. histolytica* isolated approximately four months earlier were used. Two strains were isolated from human cases of intestinal amœbiasis, two strains from human cases of cutaneous amœbiasis, one strain from a case of hepatic amœbiasis, and one strain from a case of appendicitis due to *E. histolytica*. The amœbæ were cultured regularly on a medium consisting of a solid phase of egg and whole blood (a coagulated slant) overlaid with 5 ml. of Ringer solution. Rice powder was added just before inoculation of the media. The amœba cultures were transferred three times a week and incubated at 37° C. Amœbacidal concentrations of fumagillin were determined by growing the amœbæ in culture tubes containing varying concentrations of the agent. Control tubes not containing the antibiotic were also inoculated. The tubes were read after 48 hr. of incubation by removing approximately 0·1 ml. of the sediment from each tube, placing on a microscope slide, covering with a glass cover slip, and counting the numbers of amœbæ in the low-power field of the microscope. Ten different fields were counted and average counts recorded; two samples were taken from each tube. Each experiment was performed in quadruplicate and each experiment was repeated three or four times. The concentration of fumagillin necessary to produce 100 per cent inhibition (no detectable surviving amœbæ) was determined. Resistance to fumagillin was induced by growing the amœbæ in culture media containing concentrations of the antibiotic which produced 50 per cent inhibition. The amœbæ were grown on media containing antibiotic for 2 weeks, then the susceptibility to fumagillin was re-assayed to determine the new amœbacidal end-point. Strains of amœbæ which developed resistance were afterwards cultivated in media not containing the antibiotic for 2 weeks and re-tested for their sensitivity to fumagillin in order to determine the stability of the induced resistance.

Complete inhibition of *E. histolytica* by fumagillin was obtained by concentrations ranging from 0·01 μgm. to 100 μgm./ml., depending on the strains. The two strains obtained from two different cases of cutaneous amœbiasis possessed the highest resistance to the antibiotic; concentrations of 50–100 μgm./ml. were needed to produce 100 per cent inhibition. The strains obtained from cases of intestinal amœbiasis were the least resistant to fumagillin; 0·01–0·1 μgm./ml. of fumagillin produced 100 per cent inhibition. The strains isolated from a case of hepatic amœbiasis and a case of amœbiasis of the appendix were completely inhibited by 1–10 μgm./ml. of fumagillin. The cutaneous strains, which had the highest degree of resistance, were grown in the presence of 1–10 μgm./ml. of fumagillin and re-tested for their susceptibility to fumagillin. No increase in resistance was noted. However, when the two strains from the intestinal cases which possessed the least amount of resistance, were grown in the presence of 0·001–0·01 μgm./ml. of fumagillin, the resultant cultures were highly resistant to fumagillin. These more resistant strains required 75–100 μgm./ml. before 100 per cent inhibition could be obtained. These two strains retained this induced resistance to fumagillin in spite of cultivation in the absence of the antibiotic. Sufficient time was not available to determine the permanence of this resistance. The strains which possessed intermediate sensitivity to fumagillin, namely, those obtained from the liver and the appendix, also developed resistance to the drug. However, none of the strains developed resistance to the degree that would permit growth in the presence of more than 100 μgm./ml. of the antibiotic.

It appears that resistance to fumagillin can be induced more readily in strains which are more sensitive to this antibiotic. Furthermore, the level of resistance produced was high; for example, a 1,000- to 10,000-fold increase in resistance was induced in the strains isolated from the intestine. Although this resistance was maintained for a period of one and a half months, it would be desirable to determine its permanence. No adequate explanation can be given for the failure to induce resistance in the strains which were initially more resistant to the antibiotic. It is interesting that the induced resistance of the most susceptible strains reached a maximum which was approximately the concentration required to kill the most resistant (initially) strains. It is also interesting that the resistance induced in the intestinal strains was not gradual but a sudden maximal resistance. It is conceivable that in the case of fumagillin, at least, strains of *E. histolytica* isolated from different clinical materials have different

degrees of susceptibility to this antibiotic. Admittedly, the number of strains studied could have been larger; however, the availability of amœba strains from cutaneous, brain, and extra-intestinal amœbiasis is limited.

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VIROLOGY

Effect of *p*-Fluorophenylalanine on the Multiplication of Foot-and-Mouth Disease Virus

Many amino-acid analogues are known to prevent protein synthesis. *p*-Fluorophenylalanine in particular has been shown to inhibit synthesis by competition with phenylalanine. The growth of several animal viruses is inhibited by the addition of this analogue to the growth medium (for example, the viruses of poliomyelitis[1], influenza[2], fowl plague[3] and Western equine encephalomyelitis[4]). In the case of poliovirus and influenza virus, the effect of *p*-fluorophenylalanine on the infectivity and hæmagglutinating activity, respectively, was studied. A more detailed examination of the effect of the inhibitor on the synthesis of the sub-units of fowl plague virus and Western equine encephalomyelitis virus has shown that information can be obtained on the manner in which the complete virus particles are formed. The present communication describes the effect of *p*-fluorophenylalanine on the synthesis of the infective component and viral ribonucleic acid of foot-and-mouth disease virus.

Infection of pig kidney tissue culture cells with the virus (strain 997, type *C*) leads to the rapid production of new virus in the cells (Fig. 1). The formation of the infective viral ribonucleic acid, one of the precursors of the complete virus, precedes the appearance of the virus by 15–30 min. (ref. 5). It has previously been shown[6] that the inclusion of *p*-fluorophenylalanine in the growth medium reduces the yield of virus, the extent of the reduction depending on the concentration of the analogue. Table 1 shows some typical figures for the reduction of virus titre at 4 hr. when the suspending medium was phosphate-buffered

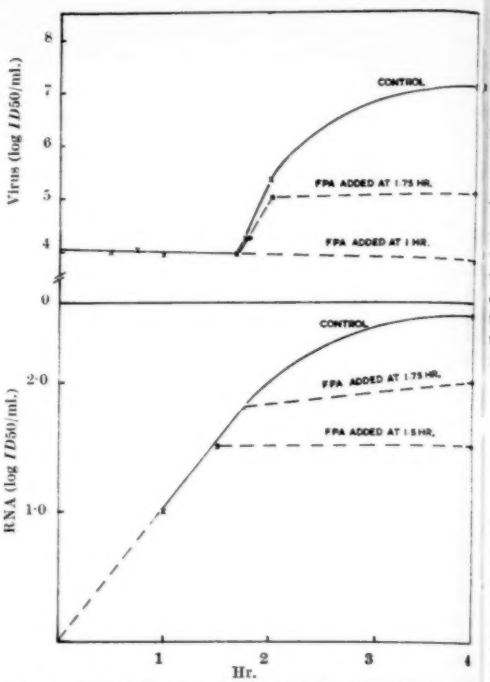

Fig. 1. Effect of adding 1,000 μM *p*-fluorophenylalanine to infected cells at different times during growth cycle (this concentration completely inhibits virus synthesis)

saline containing 0·1 per cent glucose. The effect is not due to toxicity of the *p*-fluorophenylalanine, since it can be reversed by the addition of phenylalanine[6].

As the production of foot-and-mouth disease virus depends on the prior synthesis of its infective ribonucleic acid component, it seemed to be of interest to determine the effect of *p*-fluorophenylalanine on the production of the infective nucleic acid. The figures in Table 1 show that the reduction in yield of infective ribonucleic acid obtained from infected cells inhibited by *p*-fluorophenylalanine corresponded with the fall in virus titre. It seems likely, therefore, that the formation of the precursor infective nucleic acid is in some way connected with a protein the synthesis of which is inhibited by the analogue. With phage, the early inhibition of protein synthesis prevents the formation of phage nucleic acid, since specific enzymes[7,8] must be induced to synthesize the hydroxymethyl cytosine base prior to incorporation in the nucleic acid. Once these enzymes are formed, inhibition of protein synthesis does not prevent the production of phage nucleic acid, although complete phage particles are not formed. This does not appear

Table 1. Yield of Virus and Viral Ribonucleic Acid at 4 hr. in Pig Kidney Tissue Culture Cells with *p*-Fluorophenylalanine

Concentration of *p*-fluorophenylalanine (μM)	Virus titre (mouse log *ID*50/ml.)	Ribonucleic acid titre (log *ID*50/ml.)
0	6·9	2·7
50	5·9	2·0
100	5·5	1·0
156	5·4	1·0
500	4·0	—

Titre of infected cells at time 0: $10^{4.3}$ *ID*50/ml.

to be the case with foot-and-mouth disease virus, as the addition of *p*-fluorophenylalanine (1,000 μM), even immediately before the maturation stage, leads to complete inhibition of infective virus and viral nucleic acid synthesis within 20 min. (Fig. 1). Even for natural amino-acids it has been reported that 15 min. is required for 75 per cent penetration[9], so 20 min. probably represents the time required for *p*-fluorophenylalanine to reach its site of inhibition.

It appears, therefore, that the synthesis of the infective nucleic acid of foot-and-mouth disease virus, like that of Western equine encephalomyelitis virus[4], is intimately related to the continuous synthesis of protein. This might be due to the need for the continuous synthesis of induced enzymes or to the fact that ribonucleic acid and protein synthesis are intimately coupled, as has often been postulated[10].

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Leaf Curl Virus and the Flavonoid Content of Resistant and Susceptible Strains of Cotton

Massey and Andrews[1] reported finding larger amounts of unidentified 'brown' pigments in aqueous extracts obtained from healthy cotton leaves than in those obtained from leaves infected with cotton leaf curl. By means of the known chemical tests I ascertained that the pigments are in fact anthocyanins. The work described here is a study of the changes in the amounts of various flavonoids in leaves of resistant and susceptible strains of cotton, in the course of infection.

Top leaves from two strains of Domains Sakel were used, *BAR* 14/25 as a highly susceptible strain and *X*17/30 *A* as a strain resistant to leaf curl virus. Both strains were grown in the same plot and were cultivated under identical conditions. It was observed that virus infection appeared in the susceptible strain (*BAR* 14/25) thirty days after sowing and 100 per cent infection had occurred 115 days after sowing. Infection started in the resistant strain (*X*17/30 *A*) 70 days after sowing and only 40 per cent infection was observed 120 days after sowing.

Leaf extraction and methods of determining total phenols, anthocyanins, leuco-anthocyanins, and flavanols were those used by Swain and Hillis[2].

The results (Table 1) show that the percentages of total phenols, leuco-anthocyanins and flavanols are higher in the resistant than in the susceptible strain throughout the season. Infection with the virus caused a rise in the amounts of total phenols, leuco-anthocyanins and flavanols in both strains, followed in all cases by a gradual decrease in these amounts.

Table 1. Phenolic Compounds in Cotton Leaves in the Course of Infection with Leaf Curl Virus
(Percentage on dry weight basis)

Days after sowing	Total phenols		Antho-cyanins		Leucoantho-cyanins		Flavanols	
	H	I	H	I	H	I	H	I
	BAR 14/25 (Susceptible)							
40	7·01	8·74	1·22	1·34	0·03	0·06	0·16	0·20
60	5·14	6·97	0·93	1·24	0·03	0·05	0·17	0·21
80	6·54	4·56	0·68	0·72	0·08	0·05	0·30	0·23
100	5·61	3·32	0·55	0·52	0·07	0·04	0·28	0·21
	X 17/30 *A* (Resistant)							
40	10·06	—	1·66	—	0·08	—	0·24	—
60	9·22	—	1·42	—	0·09	—	0·30	—
80	7·26	10·47	1·23	0·91	0·19	0·75	0·43	1·55
100	7·83	4·26	0·89	0·41	0·27	0·09	0·56	0·22
120	6·56	1·89	0·83	0·23	0·23	0·09	0·43	0·29

H, healthy; I, infected

The anthocyanins behaved in the same manner in the susceptible strain, that is, there was a sharp rise in the amounts following infection, then a gradual decrease, but in the resistant strain these amounts actually decreased rapidly after infection had occurred.

The results obtained in this work suggest that there might be some connexion between the flavonoid content of the leaves and the resistance of cotton plants to leaf curl virus. A study of the flavonoid content may act as a guide in spotting resistant strains in the course of breeding for resistance towards this disease.

This work was carried out in the Faculty of Agriculture, University of Khartoum; I am indebted to Dr. T. Swain of the Low Temperature Research Station, Cambridge, for his help.

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SOIL SCIENCE

Isolation of Anthraquinone from Humus

It is now generally accepted that the skeletal part of humic acids consists of heteropolycondensates of various aromatic nuclei. This concept, however, has been deduced rather indirectly from physico-chemical data such as X-ray analysis, and benzene seems to be the sole aromatic ring identified by the method of organic chemistry[1]. Recently we found anthraquinone in the decarboxylation products of the water-soluble acid fraction obtained by the alkaline permanganate oxidation of humic acids.

The humic acids used in this work belonged to so-called *A* type[2], and were obtained from a volcanic ash soil at the foot of Mt. Fuji, and from a muck soil presumably formed under the influence of volcanic ash showers on low-moor peat at Tanemori, Akita Prefecture, both soils being treated with 0·5 per cent sodium hydroxide at 100° C. for 30 min.

The sample, dissolved in *N* potassium hydroxide, was heated at about 90° C. and oxidized by adding potassium permanganate powder in small portions, until the permanganate to humic acid ratio became 4·0. The mixture was cooled, filtered to remove the precipitated manganese dioxide, and washed

thoroughly with water. The filtrate was slightly acidified with sulphuric acid and concentrated under reduced pressure.

After the removal of inorganic salts and acid-insoluble organic acids by filtration, the reddish-brown solution, adjusted to contain about 4 *N* sulphuric acid, was subjected to ether extraction for many days. The extract, composed mainly of polycarboxylic acids, was freed from ether by evaporating to dryness, and the reddish-brown solid was obtained, which was decarboxylated by the copper-quinoline method of Montgomery and Holly[3].

The distillate during decarboxylation was collected in ethyl alcohol, in which the presence of benzene was confirmed by its ultra-violet spectrum; pyridine was also detected.

The dark-coloured residue from the decarboxylation was mixed with ether, filtered through a filter paper to remove insoluble materials, and washed with hydrochloric acid (1 : 1) and 5 per cent sodium hydroxide successively to remove basic and acid fractions, respectively. The neutral fraction was then freed from ether by evaporating to dryness, and after drying in a desiccator containing silica gel, dissolved in small quantities of carbon tetrachloride.

The carbon tetrachloride solution was chromatographed through an alumina column. By developing it with carbon tetrachloride, a yellow band moved down, giving at first colourless, then yellow filtrates. Development was continued until the yellow band passed through the column and the filtrate became nearly colourless. This filtrate was passed through a silica gel column. The top of the silica gel column (fraction 3) was stained strongly reddish-brown, the other part of the column (fraction 2) colourless, and a colourless filtrate was obtained (fraction 1). Fractions 3 and 2 were scraped separately, and eluted with ether.

Three fractions could be distinguished under ultra-violet light on the alumina column after development; the upper layer (fraction 6) being dark brown in appearance and showing no fluorescence, the middle layer (fraction 5) yellowish brown and yellowish green fluorescence, and the under layer (fraction 4) grey and partly red fluorescence. These fractions were scraped out separately, and eluted with acetone. Fractions 5 and 6 were reserved for further investigation.

Fractions 1–4 were tested for aromatic compounds by examination of infra-red and ultra-violet spectra, besides routine organic analysis. The results obtained so far may be summarized as follows:

First it must be mentioned that yellow crystals were readily obtained from fractions 3 and 4 by evaporating the eluates. Needle-shaped crystals obtained by recrystallization from ethyl alcohol followed by sublimation were identified as anthraquinone, because its melting point was 285° C. and this was not depressed by mixing with authentic specimen, and the infra-red spectrum was identical with that of anthraquinone.

Fraction 1 was considered to be mainly composed of paraffins, but the ultra-violet spectrum showed small quantities of naphthalene derivatives to be present. In the case of Fuji humic acid, 2-methyl-naphthalene was confirmed by its ultra-violet spectrum and the melting point of its picrate (115–116° C.). The presence of naphthalene derivatives in fraction 2 was also presumed.

From these results, though very qualitative, it is reasonable to suppose that humic acids showing a higher degree of humification contain not only a benzene ring but also anthracene, probably in the form of anthraquinone and naphthalene rings.

Differences in the nature and properties of humic acids obtained from various soils may be explainable by the composition of their aromatic nuclei. Further investigations are proceeding.

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PSYCHOLOGY

Measuring Visual Constancy for Stationary or Moving Objects

If two objects of the same size are viewed so that one is twice as far from the eyes as the other, the retinal image of the farther object will be one-half the size of the image of the nearer object, but it does not appear half the size to the observer. They both appear almost the same size and the effect, which is due to perceptual interpretation of the retinal image, is known as size constancy[1,2].

In this method of measuring constancy of size, the eye is placed close to a small light-source which casts the shadow of an object (such as a flat disk) on a vertical screen. The angle subtended at the eye by the shadow will remain constant for any distance of the screen, but the shadow appears to expand as the screen on which it is projected is moved away from the observer. Other positions of the eye in relation to the source will produce size changes in the retinal image when the screen is moved. With the eye nearer the screen, the retinal image will contract (Fig. 1); with the eye behind the source it will expand. The degree of size constancy may be described in terms of the Thouless ratio, which is given by:

$$\log \frac{d_2 l_1}{d_1 l_2} \Big/ \log \frac{d_2}{d_1}$$

where d_1 and d_2 are distances of the screen from the eye when the shadow measures l_1 and l_2 respectively, the position of the eye being such that no size change is observed.

One can also measure constancy of shape, this being the tendency for a flat object to have a perceived

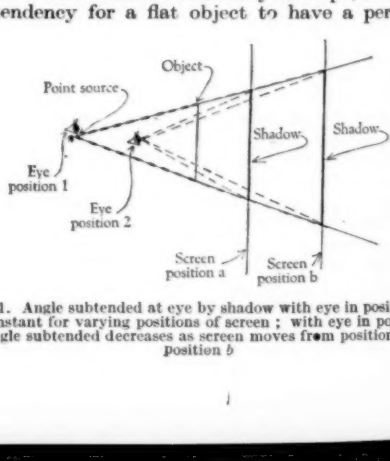

Fig. 1. Angle subtended at eye by shadow with eye in position 1 is constant for varying positions of screen; with eye in position 2, angle subtended decreases as screen moves from position *a* to position *b*

shape corresponding very nearly to its normal projection ('normal' in the geometrical sense). If the screen is rotated around its vertical axis while the shadow-casting object remains stationary, the shadow appears to enlarge horizontally. Although the shadow on the screen changes shape, the retinal image is unaltered. The shape perceived is nearly that of the shadow viewed normally.

The magnitude of this effect can be measured, again using a null method, by arranging for a compensatory change of shape of the retinal image. This may be done by rotating the (flat) object. The screen and object may be linked to provide simultaneous rotations, their relative angles of rotation being adjustable. The magnitude of the effect may again be stated in terms of the Thouless ratio, which is given by :

$$\frac{\log P - \log S}{\log R - \log S}$$

where R is the ratio of the width of the shadow on the screen to its height, P the ratio of perceived width to perceived height (arranged to be constant and measured when the screen is normal to the observer), and S the ratio of the retinal width of the shadow to the retinal width when the screen is normal. If the angles subtended at the eye are small, the Thouless ratio is given by $\frac{\log \cos p}{\log \cos q}$, where p is an angle turned through by the object and q the corresponding angle turned through by the screen (Fig. 2), the relative angles of rotation being such that no change of shape is observed.

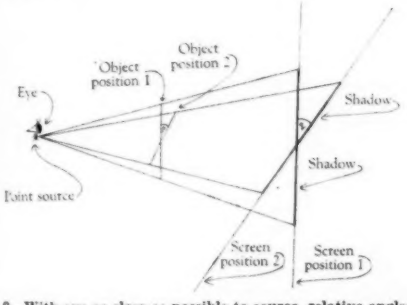

Fig. 2. With eye as close as possible to source, relative angles of rotation are adjusted until no change in shape observed

We are using this shadow method to investigate constancy during movement, which should be important in considering perceptual judgments involved in flying or driving. In particular we have tried to discover whether apparent movement due to constancy can be adapted. It is known that continuous presentation of real movement gives rise to an after-effect of apparent movement in the opposite direction[3]. The question here is: Can apparent expansion or contraction of the shadow produce an after-effect ? By projecting the shadow on a series of screens which move continuously away from the observer and are stopped after 1 min., we find that an after-effect does take place, though the retinal image of the shadow remains unchanged during the movement of the screens. During the movement the shadow appears to expand ; in the after-effect, it appears to contract. An after-effect of movement can thus be produced without shift of the image across the retina. Observation of the moving screens without the shadow does not produce the same after-effect of contraction of the shadow thrown on the screen now stationary, so the after-effect is not merely an induced movement, as described by Duncker[4].

One of us (S. M. A.) is in receipt of a Department of Scientific and Industrial Research studentship, and another (C. D. S.) of a grant from the Medical Research Council, which also provided support for apparatus.

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ANTHROPOLOGY

The Juvenile Mandible from Olduvai

FURTHER to my report in *Nature* on February 25, p. 649, about the new discoveries of hominid remains in Bed I and Bed II at Olduvai Gorge, additional information can be given on the mandible of the juvenile from the site *FLK.NN*.I, at a horizon older than that of *Zinjanthropus*.

The third lower premolar (often called the first lower premolar by anatomists) is, in all the published Australopithecines, markedly shorter mesio-distally than wide bucco-lingually. The published figures for fully erupted lower third premolars of Australopithecines in South Africa include 14 from Swartkrans with indices $(100 \times L)/B$ ranging from 76·00 to 91·9 and a mean of 83·2. Kromdrai has only one reasonably preserved third lower premolar with an index of 83·2. Sterkfontein has only two good measurable examples published, which give indices of 77·8 and 76·8 respectively. Makapan has two measurable teeth, and the indices are said to be identical, at 88·4.

It is thus clear that, on the basis of the available published data (which are confirmed by my own observations on the original material at Pretoria and Johannesburg, which Prof. R. A. Dart, Prof. P. V. Tobias and Dr. J. T. Robinson kindly allowed me to examine), the third lower premolars of all known Australopithecine specimens are broader bucco-lingually than they are long mesio-distally. The index $(100 \times L)/B$ gives a range of 76·00–91·9.

In the juvenile hominid from the level below *Zinjanthropus* of site *FLK.NN*.I the measurements of the third lower premolars are as follows :

	Length	Breadth	Index
Left	11.00	9·5	115·8
Right	11·00	9·5	115·8

Thus, while all the published length–breadth indices of fully erupted lower third premolars of all *Australopithecus* and *Paranthropus* specimens are much less than 100 (that is, broader than long) and have a range of 76·00–91·9, the indices of the tooth in the juvenile hominid from the pre-*Zinjanthropus* level are greater than 100, being in fact 115·8.

Quite apart from this very clear measurable difference which puts the third lower premolar completely out of the known range of measurable

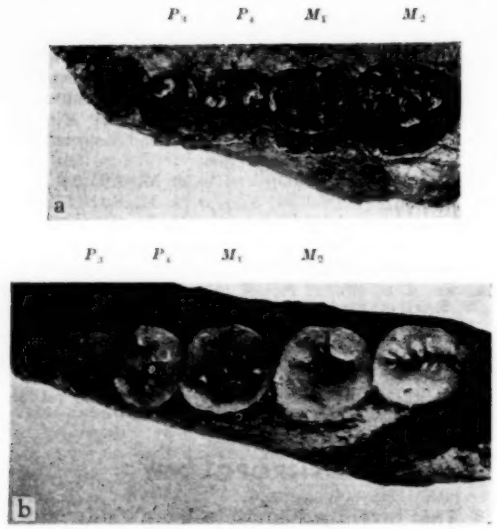

Fig. 1. Comparison between the teeth of the left side of the (*a*) pre-*Zinjanthropus* juvenile mandible with those of (*b*) an adult *Paranthropus* to show the morphological differences. In the Olduvai specimen the third molar is not present. (Not to the same scale, but reduced so that the length $P_3 - M_2$ inclusive is approximately the same so as to show relative proportions)

variation in the Australopithecines, the morphological picture is also completely different from that of any Australopithecine that I have been able to examine, or of which there are published photographs available to me (Fig. 1).

Turning next to the published data of lower fourth premolars (second premolars of some anatomists) of known Australopithecines, we find that there are 13 measurable specimens from Swartkrans, all of which are much wider bucco-lingually than they are mesio-distally. The index $(100 \times L)/B$ ranges from 61·1 to 91·1 with a mean of 82·3 (very close to the mean for the third lower premolars). Only two specimens from Kromdrai are known and they fall well within the range with indices of 84·4 and 84·7. Only two Sterkfontein lower fourth premolars appear to have been described in the literature, and these give indices of 87·0 and 82·9. I know of no measurable fully erupted fourth lower premolars from Makapan that have been recorded; the known specimens are either not fully erupted or very worn and damaged.

When we turn to the juvenile hominid from the pre-*Zinjanthropus* level at *FLK.NN*.I, we find that the fourth premolars are markedly longer mesio-distally than they are bucco-lingually. The measurements and indices are as follows:

	Length	Breadth	Index
Left	11·0	10	104
Right	11·0	10·50	104·7

Thus we find that whereas in the known Australopithecines the range in the index $(100 \times L)/B$ is from 61·1 to 91·1, in the juvenile from the pre-*Zinjanthropus* level at Olduvai, the position is quite different. The mesio-distal length is greater than the bucco-lingual width, so that the indices are more than 100.

Again, apart from measurable differences, the fourth premolars in the new jaw are morphologically different from those I have seen in the collections of Australopithecines at Johannesburg and Pretoria (Fig. 1).

The published data on the first lower molars of *Australopithecus* shows that the index $(100 \times L)/B$ varies from 114·6 in one of the Sterkfontein specimens to 100·8, also from Sterkfontein, and the indices from other sites fall within this range, both in respect of *Australopithecus* and *Paranthropus*. The first lower molars in the juvenile jaw from the pre-*Zinjanthropus* level show identical measurements of length 14·25 and breadth 12·00, giving an index of 117·00. This is outside the published range of variation of Australopithecine first lower molars. Morphologically, too, there are differences in the crown pattern, which is much simpler and more like that which can be observed in some modern Australian Aborigine teeth.

The second lower molar of the pre-*Zinjanthropus* juvenile is 16·00 mm. long and 14·00 mm. wide. In this case, the index falls within the range of variation of the known Australopithecines. Some morphological differences seem to me to be present, but need further study. I do not recall (nor have I notes on) any really comparable tooth in the collections in Pretoria or Johannesburg, but I say this with reservations.

There is a single upper molar (which appears to be an unerupted upper molar) in which the roots have not yet formed and in which the crown is completely fresh. It seems likely that it represents the third upper molar of the same juvenile as is represented by the mandible, and in which the lower second molar is erupted but scarcely worn.

If this tooth is correctly interpreted as an unerupted third upper left molar, then it confirms my view that the teeth of the juvenile pre-*Zinjanthropus* differ, in most measurable respects, from the Australopithecines. The tooth has a mesio-distal length of 13·5 and a bucco-lingual width of 12·75, giving an index of $(100 \times L)/B$ of 106, which seems to be outside the range of variation published for any Australopithecine upper third molar. Even if it is not a third upper molar but a second, it is still outside a published range, while morphologically it does not seem to be of Australopithecine type.

In conclusion, may I say that when I used the word 'child' with reference to the pre-*Zinjanthropus* juvenile in my report in *Nature* of February 25, I was not intending thereby to imply that the father was a 'man' in the sense of the accepted definition of treating him as the 'maker of tools to a set and regular pattern'. I used the word 'child' simply as meaning a juvenile of the family Hominidae.

There are stone tools and a bone tool in direct association with the juvenile, but it is not yet certain whether his family made these tools, any more than it is quite certain that *Zinjanthropus* made the tools which are in direct association with the skull from *FLK*.I.

If I am right in believing that the juvenile from *FLK.NN*.I is not an Australopithecine, but a very remote and truly primitive ancestor of *Homo*, then it is possible (and I stress the fact here that I only use the word 'possible' at this stage of the inquiry) that it was this branch of the Hominidae that also made the Oldowan tools at the site *FLK*.I where *Zinjanthropus boisei* was found. We do not yet know the final truth of this problem.

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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

BIOCHEMISTS (2) (science graduates, preferably with experience in medical biochemistry), for duties involving laboratory work in clinical pathology at Foresterhill or at Woodend Hospital—The Secretary, The University, Aberdeen (July 27).

ANIMAL PHYSIOLOGIST (with research experience), to develop work in the field of adaption of animals to environment—The Secretary, Animal Breeding Research Organization, A.R.C., Glenbourne, South Oswald Road, Edinburgh 9 (July 29).

LECTURER or ASSISTANT LECTURER IN ORGANIC CHEMISTRY—The Registrar, The University, Manchester 13 (July 31).

LECTURER IN PSYCHOLOGY at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 4).

LECTURER (preferably with research experience in surface chemistry or chemical kinetics) IN PHARMACY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 11).

LECTURER (graduate in science or engineering) IN THE DEPARTMENT OF BUILDING SCIENCE—The Registrar, The University, Liverpool (August 12).

RESEARCH ASSISTANT (with a higher degree in theoretical physics or mathematics) IN THEORETICAL PHYSICS—The Registrar and Secretary, King's College (University of Durham), Newcastle-upon-Tyne 1 (August 12).

FELLOW (specialized in organic chemistry, with special reference to the chemistry of compounds of neuropharmacological significance) IN THE DEPARTMENT OF PHYSIOLOGY, John Curtin School of Medical Research, Australian National University—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 14).

PHYSICAL RESEARCH CHEMIST (physical chemist, preferably with several years' postgraduate experience), for work concerned with fundamental studies on gelation—The Director of Research, British Gelatine and Glue Research Association, 2a Dalmeny Avenue, London, N.7 (August 14).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF APPLIED MATHEMATICS—The Registrar, The University, Liverpool (August 15).

LECTURER/SENIOR LECTURER (preferably with research interests in epidemiological or immunological aspects) IN PARASITOLOGY in the Faculty of Rural Science, University of New England, Armidale, New South Wales, Australia, to teach and carry out research in parasitology with particular reference to livestock aspects—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 15).

RESEARCH ASSISTANTS (2) (with at least a B.Sc. degree or equivalent, and a special interest in physical chemistry or chemical engineering) IN THE DEPARTMENT OF CHEMISTRY, to continue a study of the equilibrium properties of imperfect gases under the terms of a contract between the University and the D.S.I.R.—The Registrar (Room 222, O.R.B.), The University, Reading (August 15).

SENIOR TECHNICIAN or TECHNICIAN (with the A.I.S.T. or equivalent qualification) IN THE DEPARTMENT OF ZOOLOGY, University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 15).

TEXTILE TECHNOLOGIST (man or woman, with a first- or second-class honours degree in textile technology, chemistry or physics, or Associate or Fellow of the Textile Institute, or equivalent qualifications) at the Tropical Products Institute, London, W.C.1, to supervise a group concerned with research into, and laboratory examination of, various aspects of animal and vegetable fibres, pulp, paper and board-making materials, hides, skins, etc., of tropical origin—Civil Service Commission, 17 North Audley Street, London, W.1), quoting Ref. No. S 5333/61 (August 15).

LECTURER or ASSISTANT LECTURER (with experience and research interests in plant physiology) IN BOTANY at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 20).

LECTURER IN MECHANICAL ENGINEERING—The Registrar, Trinity College, Dublin 2 (August 28).

READER IN AERONAUTICS at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 29).

DIRECTOR OF THE NATURAL HISTORY MUSEUM, Adelaide, South Australia—The Agent-General for South Australia, 56 Strand, London, W.C.2 (August 31).

KEEPER OF SCIENTIFIC BOOKS (with considerable experience of university or research library administration and a good honours degree preferably in science), to superintend the Radcliffe Science Library—The Librarian, Bodleian Library, Oxford (August 31).

LECTURER IN PHILOSOPHY at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, August 31).

LECTURERS (3) IN CHEMISTRY at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

READER (with a special interest in the properties of materials) IN ELECTRICAL ENGINEERING at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 31).

LECTURER IN THE DEPARTMENT OF MEDICAL PHYSICS—The Secretary, Royal Free Hospital School of Medicine (University of London), 8 Hunter Street, London, W.C.1 (September 1).

SENIOR LECTURER IN APPLIED ELECTRONICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 1).

LECTURER or ASSISTANT LECTURER (medically or non-medically qualified candidate) IN THE DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/N (September 4).

LECTURER or JUNIOR LECTURER IN PURE MATHEMATICS—The Registrar, Trinity College, Dublin 2 (September 4).

LECTURER IN SOCIAL ANTHROPOLOGY at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, September 8).

LECTURER/SENIOR LECTURER (well qualified in electrical engineering or physics, general experience in electronics and a considerable detailed knowledge of solid state devices and circuits) IN ELECTRICAL ENGINEERING at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

SENIOR LECTURER IN PHYSICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, September 30).

ASSISTANT LECTURER (with an honours degree or an honours diploma in technology in applied physics, and preferably research or industrial experience in some branch of applied physics) IN PHYSICS—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

BIOCHEMIST (with a first- or second-class honours degree or graduate member of the Royal Institute of Chemistry), for duties in the Hæmatological Laboratory—The House Governor, King's College Hospital, Denmark Hill, London, S.E.5.

LECTURER or ASSISTANT LECTURER (particularly interested in petrology and mineralogy) IN GEOLOGY—Prof. D. Williams, Geology Department, Imperial College of Science and Technology, London, S.W.7.

LECTURER (preferably with a higher degree and research experience in an industrial environment) IN METALLURGY in the Department of Materials—The Recorder, College of Aeronautics, Cranfield, Bletchley, Bucks.

LECTURER (university graduate in physics with industrial and/or research experience in radiation protection or a kindred subject, and preferably some teaching experience) IN PHYSICS—The Clerk to the Governing Body, Borough Polytechnic, Borough Road, London, S.E.1.

PHYSICISTS or ELECTRICAL ENGINEERS (with experience in one or more of the following fields: high vacuum; R.F. techniques (75 Mc./s.); high power rectifier and pulse circuits; or general electronic instrumentation and controls), to assist with the construction and commissioning of a large heavy ion linear accelerator—The Assistant Director, Linear Accelerator Laboratory, Physics Department, The University, Manchester 13.

POSTGRADUATE STUDENT (recent graduate with a good degree in physics or physical chemistry) IN THE DEPARTMENT OF PHYSICS, to research under the supervision of Dr. J. G. Powles on some aspect of magnetic resonance—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1.

RESEARCH ASSISTANT (recent graduate in chemistry with an interest in zoology or comparative biochemistry), to join a team investigating chemical constituents of the biles of many species, with a view to a fuller understanding of evolutionary theory and taxonomic principles—Prof. G. A. D. Haslewood, Biochemistry and Chemistry Department, Guy's Hospital Medical School, London Bridge, London, S.E.1.

RESEARCH ASSISTANT (recent physics graduate) IN THE DEPARTMENT OF PHYSICS, for track analysis in connexion with the National Bubble Chamber project—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15.

SCIENTIFIC OFFICER (with a first- or second-class honours degree in chemical engineering or in chemistry, and postgraduate training or experience in chemistry) at the Humber Laboratory, Kingston-upon-Hull, Yorkshire, to work on problems of both fundamental and applied research concerned with fish-smoking; the transport of fresh and frozen fish; and the manufacture of fish meal—The Director, Torry Research Station, D.S.I.R., Aberdeen, quoting Ref. Torry 8Z.

SENIOR LECTURER (graduate in pharmacy or chemistry with an established interest in the medicinal field) IN MEDICINAL CHEMISTRY at the Department of Pharmacy—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C.1.

SENIOR LECTURER (with a good university degree in agriculture or veterinary science, considerable postgraduate experience in research and/or teaching in a higher institution, and conversant with tropical work in animal husbandry) IN ANIMAL HUSBANDRY in the School of Agriculture, Kumasi College of Technology, Ghana—The Assistant Registrar, Kumasi College of Technology, 29 Tavistock Square, London, W.C.1.

SENIOR RESEARCH ASSISTANT (with a Ph.D. or appropriate research experience) IN THE DEPARTMENT OF PHYSICS, The University, Leeds 2.

SENIOR RESEARCH ASSOCIATES (2) (experienced electrical engineers or physicists) IN THE DEPARTMENT OF PHYSICS, for the development of the proton synchrotron—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15.

TECHNICAL OFFICER (with a degree or National Diploma in Agriculture or a degree in agricultural botany), to assist in work on herbage seed production—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

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